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ORGANOPHOSPHATE INSECTICIDE TOXICITY IN RAINBOW TROUT (*Salmo gairdneri*) : EFFECTS OF TEMPERATURE AND INVESTIGATIONS ON THE SITES OF ACTION

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Doctor of Philosophy

by

Maitree Duangsawasdi

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a Ph.D. thesis entitled: Investigations on the Effects of Temperature and the Sites of Organophosphorus Insecticides in Rainbow Trout (Salmo Gairdneri) ....

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BY

MAITREE DUANGSAWASDI

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

In order to protect fish from organophosphorus (OP) insecticide applications, field monitoring programs for assessing the effects of OP insecticides on fish have to be developed. Detection of OP insecticide pollution in natural water requires knowledge of the sites of action and the effects of environmental factors on the toxicity of these chemical in fish. Two OP insecticides; acephate (a phosphoramidothioate and a direct inhibitor of cholinesterase [ChE]) and fenitrothion (a phosphorothioate and an indirect inhibitor of ChE), were tested on rainbow trout (Salmo gairdneri) fingerlings to study the effects of temperature stress on acute lethality and ChE inhibition in brain and skeletal muscle. Physiological responses of cardiovascular and respiratory systems, ChE inhibition in various tissues and changes in serum electrolytes in adult fish exposed to each insecticide were observed to provide some more understanding on the sites of action of OP insecticide producing death in fish.

Temperature stress affected the acute lethality of each insecticide but was more pronounced with fenitrothion than with acephate during the first 24 hour period. The effects of temperature stress became less after 48 hours and no significant effects were observed after 96 hours of exposure. Expressed as LC_{50} values (the concentration that produced 50 percent mortality), the toxicity of fenitrothion was about 600 to 1000 times greater than acephate depending upon test temperature. There was no correlation between ChE inhibition levels in the brain and skelet muscle of rainbow trout fingerlings and the concentration of acephate

and fenitrothion which produced mortality.

Acephate and fenitrothion both produced a decrease in heart rate, increase in ventilation rate and amplitude in adult rainbow trout. Fenitrothion produced an increase in cough frequency, but acephate did not. Acephate and fenitrothion produced differential patterns of ChE inhibition in various tissues of fish. The extent to which this enzyme was inhibited depends on the physicochemical properties and probably the distribution within the fish body of both insecticides. ChE activities in the tissues of cardiovascular and respiratory systems especially gills, heart and serum were inhibited to a greater extent than brain and skeletal muscle by each insecticide. It is suggested that these two systems are adversely affected by OP insecticides and therefore could be used to detect exposure to OP insecticides in fish. Acephate and fenitrothion produced changes in serum electrolytes characterized especially by an increase in serum potassium and a decrease in serum chloride concentrations. These changes were considered to be caused by the movement of electrolytes among fluid compartments to maintain electroneutrality.

This study indicates that the cardiovascular and respiratory systems in fish are very important sites of action for OP insecticide toxicity, and that this toxicity depends on physicochemical properties, eg. lipid solubility and degree of ionization of the insecticide, and on environmental factors, eg. temperature.

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INTRODUCTION

Organic compounds containing phosphorus are essential constituents of protoplasm and play important roles in the maintenance of life. On the other hand, many organophosphorus (OP) compounds are artificially produced for use as lubricants, oil additives, plasticizers, and pesticides (Eto, 1974). The discovery of the insecticidal action of these compounds was made in Germany during the Second World War from efforts directed toward development of chemical-warfare agents (O'Brien, 1967). In addition to insecticidal activity, a variety of other biological activities of OP compounds was discovered. For example, these compounds are used as acaricides, nematocides, anthelmintic agents and herbicides. It is surprising that such great varieties of chemical, physical and biological properties of these OP pesticides are governed by the selection of groups attached to the phosphorus atom.

Owing to the relatively low persistence and high effectiveness of OP pesticides, their application to agriculture, public health, and related fields has been growing rapidly in many countries. About 140 organophosphorus compounds are now used as pesticides and more than 60,000 tons a year of OP pesticides are produced in the United States alone (Eto, 1974).

Mechanism of action of OP insecticides in vertebrates

The insecticidal activity and mammalian toxicity of OP insecticides are generally believed to be due to the inhibition or inactivation of cholinesterases (ChE) which is a group of the hydrolytic enzymes for acetylcholine (ACh), a nerve transmitter, released in the process of cholinergic transmission. ACh is a neuro-transmitter operates in

cholinergic synapses which include synapses in the central nervous system eg. in brain and spinal cord. ACh also operates in the synapses of the peripheral somatic nervous system eg. the neuromuscular junction of the motor nerves, sensory nerve endings of skeletal muscle, and also in the autonomic nervous system eg. all preganglionic, postganglionic and a few postganglionic sympathetic synapses which consist of nerves, ganglia and plexuses that provide the innervation to the heart, blood vessels, glands, viscera and smooth muscle (Koelle, 1970a). The inhibition of ChE by OP insecticides, therefore, disturbs normal nervous function and finally results in the death of animals.

Knowledge of the involvement of ChE in the areas of neurophysiology (Ruch and Patton, 1965), neurobiochemistry (Silver, 1974) and neuropharmacology (Koelle, 1970a, 1970b) is extensive. These areas are well documented in the above text.

ChE inhibition in fish by OP insecticides

Experiments have shown that fish exposed to sublethal and lethal concentrations of several OP insecticides exhibited a reduced level of ChE activity in excised brain tissue and the surviving fish removed from exposure to OP insecticide demonstrated a capacity for regeneration of this enzyme (Weiss, 1958; 1959). However, brain ChE inhibition in fish exposed to OP insecticides showed a broad range of response. The brain ChE activity of fishes that died from exposure to OP insecticides ranged from zero activity to 98.6 percent of normal level (Weiss, 1961). Fish surviving such exposures had brain ChE activity as low as 5.4 to 10 percent of normal level (Weiss, 1961; Gibson et al 1969). These investigators suggested that death usually occurs when fish brain ChE

activity is 40 to 70 percent inhibited as compared to that of non-exposed, control fish of the same species. Coppage (1972) and Coppage and Mathews (1974) suggested 80 percent inhibition of the ChE activity of fish brain is the critical level in short term OP insecticide poisoning. These investigators also concluded that the degree of inhibition of brain ChE activity by OP insecticides is a function of concentrations of the insecticides, exposure time, specific chemical nature of insecticides, water chemistry conditions and fish species.

Inhibition of brain ChE in fish has been proposed as a means of detecting OP insecticide pollution in natural waters and has been used for monitoring purposes (William and Sova, 1966; Holland et al 1967; Coppage and Braidech, 1976). Nicholson (1967) suggested that a 10 percent depression of ChE concentration in fish brain should be used as an upper limit for evaluating water quality relative to OP insecticide contamination. Gibson et al (1969) reported that mortality and recovery from OP insecticide poisoning in fish are not necessarily related to the degree of ChE inhibition in the brain. The degree of ChE inhibition in brain has also been reported as not being correlated with the decline in behavioral and adaptive responses in fish (Rosic et al 1974).

Many studies have been done on the effect of anti-ChE agents on the neuromuscular system in other vertebrates, but only recently has the neuromuscular system in fish received attention. Pharmacological investigations of neuromuscular transmission in fish have been reported by Hidaka and Kuriyama (1969), Diamond and Mellanby (1971), and Mellanby and Thompson (1972). They reported that the major lethal action of

anti-ChE agents was a blocking of neuromuscular transmission by preventing both nerve stimulated and spontaneous release of ACh from presynaptic terminals. Schneider and Weber (1975) evaluated the significance of ChE to neuromuscular transmission in the pectoral fin abductor muscle of largemouth bass (Micropterus salmoides) and reported the occurrence of ChE inhibition by an OP insecticide, DFP (diisopropylfluorophosphate). They concluded, however, that the acute toxic effects of DFP to largemouth bass are not mediated by a collapse of neuromuscular function.

Fish skeletal muscle has also been suggested as a useful tissue as fish brain in ChE inhibition study in detecting exposure to OP insecticides and could facilitate sampling for enzyme assays especially with small fish where brain dissection may be difficult (Benke and Murphy, 1974).

Metabolism of OP insecticides in fish

OP insecticides can be separated into 2 major groups, according to whether they are activated, as direct inhibitors and indirect inhibitors (Loomis, 1974). The majority of OP insecticides are indirect inhibitors, eg. phosphorothioates, which have the sulphur atom linked to the central phosphorus atom (P=S) as the basic structure. These compounds have little, if any, direct inhibitory activity of ChE and are activated by the mixed-function oxidase (MFO) enzyme systems in liver to the more potent inhibitors, eg. the oxygen analogs (P=O). This enzyme system is one of the major metabolic systems available for eliminating foreign compounds such as drugs, petroleum products and insecticides (Chambers and Yarbrough, 1976). Although these metabolic reactions are most often detoxications, there are some reactions, especially epoxidation and

desulfuration, which can be activations to more toxic compounds (Chambers and Yarbrough, 1976).

The ability to metabolize OP insecticides and the fact that the toxicity of OP insecticides is related to hepatic metabolic activity has been demonstrated in a wide variety of fish species (Buhler and Rasmusson, 1968). The activation of the indirect inhibitors, eg. the phosphorothioate (P=S) compounds, to the active ChE inhibitor by liver preparations has been observed in brook trout (Salvelinus fontinalis), brown trout (Salmo trutta), pumpkinseed (Lepomis gibbosus), black bullhead (Ictalurus melas), winter flounder (Pseudopleuronectus americanus), and shorthorn sculpin (Myxocephalus scorpius) (Potter and O'Brien, 1964; Murphy, 1966). Liver of pumpkinseed sunfish can both activate and detoxify parathion and methyl parathion (phosphorothioate compounds), but at a slower rate than mouse liver (Benke et al. 1974). Sesamex, a MFO inhibitor, prevented the activation of parathion in mosquitofish (Gambusia affinis) and increased the 48 hr LC_{50} value (the concentration of insecticide that produced 50 percent mortality at 48 hours of exposure) by almost 11-fold (Ludke et al. 1972). A 57 percent inhibition of brain ChE in the sesamex-treated fish as compared to 89 percent inhibition in the non-treated groups of the same parathion concentrations was reported. Activation of parathion by MFO, as indicated by brain ChE inhibition, was also noted in golden shiners (Notemigonus crysoleucas), green sunfish (Lepomis cyanellus), and bluegill sunfish (Lepomis macrochirus) (Gibson and Ludke, 1973).

Effects of temperature on fish and OP insecticides

The effects of temperature on fish are profound. From enzymatic

reactions through hormonal and nervous control to digestion, respiration, osmoregulation and to all aspects of performance and behaviour, fish are influenced by temperature. Temperature always act as a controlling factor, and may at certain levels act as a directive or lethal factor for fish (Fry, 1967).

Temperature affects the Michaelis-Menten constant, Km (a substrate concentration for enzyme at which the velocity of reaction is half maximal), but the relationship between temperature and Km is quite complex. In general, over an upper temperature range, the Km varies directly with temperature; at lower thermal extremes the effects of temperature are often reversed (Somero, 1969; Fry and Hochachka, 1970). Brain ChE activity of killifish (Fundulus heteroclitus) varies inversely with the temperature of acclimation (Baslow and Nigrelli, 1964). Hazel (1969) however, reported that the specific activity of ChE in brains of goldfish (Carassius auratus) and killifish (Fundulus heteroclitus) acclimated to 25 C was significantly higher than in fish acclimated to 5 C. Increase in ChE activity with environmental temperature is also observed in brain tissue of bluegills (Lepomis macrochirus) (Hogan, 1970).

A rapid increase in temperature imposes a stress on fish which is exhibited by hyperglycemia, hypocholesterolemia, increased blood hemoglobin and decreased interrenal ascorbic acid (Wedemeyer, 1969; 1973). Acute and moderate thermal shock in rainbow trout (Salmo gairdneri) is accompanied by decreases in plasma sodium and chloride levels, a decrease in tissue water, and an increase in extracellular fluid (Reaves et al. 1968).

A temperature rise in an aquatic environment will cause the rates of many biological processes in fish including swimming activity (Brett, et al. 1958), digestion (Hathaway, 1927) and respiration (Hughes and Roberts, 1970) to increase. The term Q_{10} is a factor by which the reaction rate is increased by a temperature increase of 10 C (Warren, 1971). In general, Q_{10} values associated with physical processes, such as diffusion or conductivity, and those associated with photochemical reactions are less than 1.5, while Q_{10} for thermochemical (enzymatic) reactions range from 2 to 3 but can vary widely because the Q_{10} will depend on the thermal history and normal temperature range of a fish (Hoar, 1975).

With increases in temperature, toxicities of some substances are increased and the resistance to disease in fish is lowered (Jones, 1964). Toxicity of insecticides to fish is generally thought to be greater at higher temperatures. Macek et al.(1969) studied the effects of temperature on the susceptibility of bluegills (Lepomis macrochirus) and rainbow trout (Salmo gairdneri) to selected pesticides. They found an increase in the susceptibility of fish to most pesticides tested as temperature increased. They suggested that a probable mechanism involved is a higher rate of pesticide uptake at the higher temperature than at lower temperature by an indirect effect of temperature on metabolism. Increased temperature may also decrease toxicity of some pesticides to fish as observed with DDT and methoxychlor, chlorinated hydrocarbon insecticides, but the mechanism involved is still not clear (Johnson, 1968; Macek et al. 1969). Increasing temperature can accelerate not only the penetration and harmful action of insecticides but also

adaptive responses, including their elimination from the body (Wilber, 1969).

Temperature can also affect the metabolic processes of OP insecticides including biotransformation or activation by MFO enzyme system in fish liver (Chambers and Yarbrough, 1976). The increased susceptibility of fish to a pesticide could be related to an increased level of enzymatic activity at higher temperature than at lower temperature. Macek et al. (1969) suggested that the observed increase in the susceptibility of rainbow trout to Dursban, a phosphorothioate OP insecticide, as temperature increases is apparently related to an increase in the activation process of Dursban to its phosphate analog which is more toxic.

Sites of action of OP insecticides in fish

The target sites of action for OP insecticide acute lethal intoxication in mammals are well understood (Koelle and Gilman, 1949; Holmstedt, 1959; Koelle, 1970b). The cause of death in mammals is primarily respiratory failure, usually accompanied by a secondary effect on the cardiovascular system (Koelle, 1970b). Respiratory failure is caused by peripheral paralysis of the diaphragm owing to the blockage of neuromuscular transmission and by a disturbance of the respiratory center in the medulla oblongata of the brain resulting in hypoxia (Koelle, 1970). In poikilotherms, especially fish, the target organs and sites of action of OP insecticides are still not known.

Cardiovascular and respiratory systems of fish are controlled by the central and peripheral nervous systems as in other vertebrates (Campbell, 1970; Randall, 1970). The pacemaker of the fish heart is

normally located in the sinus venosus or at the junctional area between the sinus venosus and the atrium and is innervated by the cardiac branches of the vagus nerve (Randall, 1966). It was suggested that the vagal inhibitory effect on fish heart was essentially the same as that on the amphibian and mammalian hearts, and that the cardio-inhibitory effect is mediated by an cholinergic innervation (Cobb and Santer, 1973; Saito, 1973). ACh at low concentrations is reported to reduce the heart rate of the eel (Anguilla japonica) and the effect is abolished by atropine, a cholinergic blocking agent. The inhibition of ChE in the heart muscle by OP insecticides has been reported in mammals (Holmstedt, 1959; Sharma et al. 1973) but no data are available on the inhibition of ChE in fish heart muscle.

The heart must be supplied with sufficient oxygen and metabolic fuels to replace continuously the energy expended both as useful work and as energy lost because the heart is less than perfectly efficient as a pump (Nasser, 1970). The placement of the teleost heart far downstream of a single-loop circulation presents a problem of oxygen supply. Two sources of oxygen are available to it: a high volume, low concentration supply of venous blood going through the lumen; and a low volume, high-concentration arterial blood diverted from the dorsal aorta to the heart through the coronary arteries (Cameron, 1975). Myocardial ischemic conditions may occur whenever the coronary blood flow is insufficient in relation to oxygen requirement of the myocardium. The small quantity of oxygen contained within a given mass of water compared with the same mass of air clearly imposes a limit on the range of respiratory homeostasis in fish and therefore fish are in a greater danger

of hypoxia than land vertebrates (Satchell, 1971).

ChE activity in whole or fraction of human blood has been routinely used for a number of years as an indicator of exposure to anti-ChE agents (Gage, 1967). Significant inhibition of ChE activity within either plasma or red blood cells indicates exposure to an inhibitor of ChE (Witter, 1963; Wills, 1972). The preparation and purification of ChE in serum and erythrocyte of carp (Cyprinus carpio) and its chemical properties are reported by Kuwabara and Hayama (1961) and the inhibition of ChE in both serum and erythrocyte by OP insecticides is reported by Hayama and Kuwabara (1962). Investigation of the ChE characteristic in the blood to assess some of the effect of OP insecticides in other fish species is also reported in channel catfish (Ictalurus punctatus) (Hogan, 1971). Serum ChE activity of rainbow trout (Salmo gairdneri) is found to be more sensitive than brain ChE activity as an indicator of sublethal poisoning by fenitrothion, an OP insecticide (Lockhart et al. 1973).

Since the respiratory and circulatory systems of fish are intimately related, investigations on the effect of OP insecticides on physiological function of fish gills could provide useful information. The gills of freshwater teleosts function as the primary site for the active transport of ions or materials from the external media and for the respiratory exchange of gases. Therefore, any substances that interfere with gill functions will affect the homeostatic condition of the fish body. Gill functions in fish are underneural control and are regulated by autonomic nerve fibers in the gill (Campbell, 1970). The effects of cholinergic and adrenergic drugs on gill function and histological studies demonstrate the presence of nerve endings on the pillar cells and filamental vessels

of the gills (Ostlund and Fange, 1962; Rankin and Maetz, 1971; Randall et al. 1972). It is suggested that fish regulate vascular resistance as in higher vertebrates (Satchell, 1971) and ACh increases filamental sinus blood flow while epineprine increases secondary lamella blood flow (Richards and Fromm, 1969). The functional surface area of rainbow trout gills can be regulated by changing perfusion pathway of the blood flow and ACh decreases the functional gill surface area and increases the overall branchial vascular resistance (Bergman et al. 1974). Several insecticides, including OP, decrease the rate of fluid flow through isolated perfused rainbow trout gills which indicates that resistance to fluid flow through gills is increased (Fromm et al. 1971).

OP insecticides used in the study

Fenitrothion (O,O-dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate) an indirect ChE inhibitor, is a broad spectrum OP insecticide used extensively throughout the world for control of agricultural and forest pests (NRCC, 1975). It has low toxicity to mammals and has been used as a replacement for DDT in Canadian forests to control the spruce budworm since 1969 (NRCC, 1975). In Canada, annual spray operations at the dosage of 2-4 ounces/acre range have involved millions of acres of forest (NRCC, 1975). Aerial spraying of fenitrothion causes the aquati environment, existing within the forests, to be contaminated at levels which produce lethal and sub-lethal effects to fish species (NRCC, 1975 Fish mortalities, in and adjacent to areas after aerial spray of fenitrothion, have been reported (Hatfield and Riche, 1970; Kingsbury, 1973; Coté and Tétreault, 1973). Determination of ChE activity in the brain of atlantic salmon parr (Salmo salar) (Zitko et al. 1970) and in

whole body of atlantic salmon fry (Wildish et al. 1971) exposed to known concentrations of fenitrothion established that this compound inhibited ChE in fish. Klaverkamp et al. (1975) studied the acute lethality of fenitrothion to rainbow trout fingerlings and reported the 24 hr LC_{50} as 3.8 - 4.6 mg/L. Zitko and Cunningham (1975) reported the toxicity of fenitrothion to juvenile Atlantic salmon (Salmo salar) at 47 and 92 hour periods of 2.50 and 1.25 mg/L respectively. Other investigators reported the LC_{50} values in various fish species in the range between 2.0 mg/L at 24 hours of exposure to 1.0 mg/L for 96 hours of exposure (Sprague, 1966; Bull, 1971; Hatfield and Anderson, 1972).

Many investigators have reported behavioural and activity changes in various species of fishes exposed to or fed with fenitrothion at sublethal concentrations. Learning ability to atlantic salmon parr (Salmo salar) is completely inhibited after 24-hour exposure to fenitrothion at 1.0 mg/L (Hatfield and Johansen, 1972) and the parr are more vulnerable than before exposure to predation by large brook trout (Salvelinus fontinalis) (Hatfield and Anderson, 1972). The suppression of hierarchical behaviour is reported in brook trout when fed with 10 mg fenitrothion/gm of food (Wildish and Lister, 1973). Swimming speed of brook trout is decreased when fish are exposed to fenitrothion at concentrations of 0.5 to 1.5 mg/L (Peterson, 1974). Feeding behaviour of coho salmon (Oncorhynchus kitsutch) is depressed and fish are unable to mainta position after 2 hour of exposure to 0.75 mg/L fenitrothion (Bull and McInerney, 1974). Most of these investigators have suggested various patterns of behavioural changes to indicate physiological impairment in fishes exposed to sublethal levels of fenitrothion. However, they cannot identify the specific behavioural parameters, which are sensitive

indicators of fenitrothion and other OP insecticides, but believe that the physiological impairments are related to ChE inhibition.

Acephate (O-S-dimethyl acetylphosphoramidothioate), a direct ChE inhibitor, is a relatively new OP insecticide and has been tested in the laboratory as a potential insecticide against forest pests in Canada (Nigam and Hopewell, 1973). Acephate is a very promising systemic insecticide and is the N-acetyl derivative of the OP insecticide, metamidophos. The N-acetyl results in a decrease in mammalian toxicity (Eto, 1974). Acephate showed effectiveness equal to fenitrothion for control of spruce budworm larvae and it was therefore recommended for aerial spray trials against budworms in 1974 (Hopewell and Nigam, 1974). It has lower acute lethal toxicity than fenitrothion to mammals, birds and fish (Chevron Co., 1973).

Only a few studies have been conducted on acephate toxicity in fish. The acute lethality of acephate to various species of fish for 96 hours ranges from 1,700 to 9,500 mg/L (Chevron Co., 1973). The 24 hr LC_{50} of acephate to rainbow trout fingerlings is 900-1050 mg/L (Klaverkamp et al. 1975). No data are available on the effect of acephate on ChE inhibition in fish.

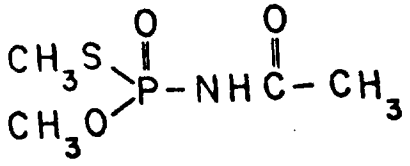
The chemical structures of acephate and fenitrothion are shown in Figure 1.

Statement of the problem and objectives of the study

Fenitrothion, a lipid-soluble OP insecticide and an indirect ChE inhibitor, and acephate, a water-soluble OP insecticide and a direct ChE inhibitor are being used extensively in Canada and in other countries for controlling agricultural and forest pests. It is evident that the

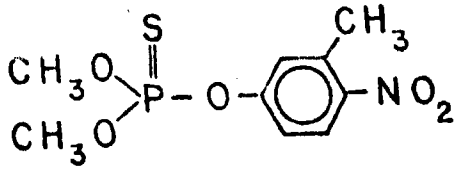
Figure 1. Chemical structures of acephate and fenitrothion.

ACEPHATE



O,S-DIMETHYL ACETYLPHOSPHORAMIDOTHIOATE

FENITROTHION



O,O-DIMETHYL-O-(3-METHYL-4-NITROPHENYL)-
-PHOSPHOROTHIOATE

aquatic environment is contaminated from the application of both insecticides which in turn produce effects on fish species.

There are large temperature changes in the aquatic environment within Canada and other countries. These changes in temperature may, in turn, influence the toxicity of OP insecticides to fish. There are very few data, if any, on the effects of environmental factors, especially temperature, on the toxicity of fenitrothion and acephate to fish.

One of the purposes of this study was to investigate the effects of temperature stress on the toxicity of fenitrothion and acephate and on ChE inhibition in brain and skeletal muscle in rainbow trout (Salmo gairdneri).

The rates of insecticide absorption, distribution, metabolism and excretion in fish are dependent on temperature. The effects of temperatur stress on toxicity of indirect ChE inhibitors (ie. fenitrothion) should be more complicated than that of direct ChE inhibitors (ie. acephate) since at least two or more enzyme reactions are involved, ie. the activati process by liver enzyme systems and reaction with target enzyme (ChE). Therefore, the rate of toxicity of fenitrothion is expected to be more dependent on temperature than acephate.

The sites of action of OP insecticides in mammals are well understood, however, the physiological target sites in fish are still unknown. Most physiological studies on the effect of OP insecticides on fish have investigated ChE inhibition in brain and skeletal muscle since these are thought to be the target organs in mammals. Very few investigations have been directed toward broadening the basic understanding of the ChE inhibition by OP insecticide in different fish tissues.

Another purpose of this study was to investigate the physiological

target sites of action of fenitrothion and acephate in rainbow trout by observing the responses of heart rate, respiration rate and amplitude and cough frequency; and the ChE inhibition in various tissues of the cardiovascular/respiratory systems; and changes in serum electrolyte concentrations.

Since it appears that the cardiovascular and respiratory systems of fish are affected by substances that cause ChE inhibition in the nervous system, investigation of physiological responses and ChE inhibition in various tissues of both systems may help to identify the sites of action of OP insecticides.

This study may provide more useful information on the effects of temperature on OP insecticide toxicity and on the sites of action that produce toxicity in fish of two types of OP insecticide; lipid-soluble and indirect ChE inhibitor represented by fenitrothion, and water-soluble and direct ChE inhibitor represented by acephate. This information is needed for the development of OP insecticide pollution control and monitoring programs for protecting aquatic life.

MATERIALS AND METHODS

1. Effects of temperature on acute lethality and ChE inhibition

1.1 Fish holding and feeding conditions

Fish used in this study were rainbow trout (Salmo gairdneri) fingerlings of either sex with a mean weight of 8.85 grams (range, 4.90 - 15.40; standard deviation, 2.30) and a mean length of 8.64 centimeters (range, 7.20 - 11.00; standard deviation, 1.21). They were furnished by The Balmoral Hatchery, Department of the Environment, Manitoba. The fish were transported from the hatchery and were held at 10°C in the wet lab at the Freshwater Institute, Winnipeg, Manitoba until required for the experiment.

Fish were transferred from the wet lab to the acclimation room and were held at 15°C in a 200 liter fiberglass holding tank that received a continuous flow of dechlorinated Winnipeg City tap water (hardness as $CaCO_3$=90 mg/L, conductivity = 190 µmho/cm, pH = 8.0) at the rate of 6 liters per minute. Dechlorination was accomplished by passing tap water through an electronic water sterilizer (Armstrong and Scott, 1974) using ultraviolet lamps (Aquafine Sterilizer Model CSL-8, Aquafine Corporation, U.S.A.).

The center of each tank was equipped with two standpipes, concentrically placed (outer standpipe open at the bottom, inner standpipe open at the top), allowing the feces which accumulated on the bottom to flow through the standpipe system to the drain.

Water in the tank was continuously aerated through an air stone. Water temperature in the tank was maintained constant at 15°C, which varied less than ±1°C, by a temperature control unit equipped in the tank

as described by Harrison et al.(1975). Holding tanks were illuminated with fluorescent lights, which were electrically switched on and off with a timer to keep a photoperiod cycle of 12 hours day-light and 12 hours darkness.

The fish were fed once daily on a diet of dry pellet trout food (EWOS No. 3 granule Trout Starter, Astra Chemical Ltd., Ontario, Canada) at the rate of 0.26 gm of food per day per fish or about 3 percent of body weight. Fish were maintained in very good health on this diet and no significant mortality occurred. Feeding was stopped 48 hours before starting the experiment. The fish were allowed to acclimate to these holding conditions for at least 3 weeks prior to use in the study.

1.2 Experimental conditions

1.2.1 Test vessels: The test vessels were circular polyethylene tanks, 23 cm in diameter and 20 liters in volume. Eleven test vessels were placed in a temperature controlled water bath (Harrison et al. 1975). Clean plastic bags were placed in the test vessels at the start of each experiment, to prevent residual contamination of the test vessels between experiments and, when secured at the top, to prevent fish from jumping out of the test vessels. Plastic bags were discarded after each experiment. The test vessels were rinsed with dilution water for 24 hours before fish were introduced to the test vessels. Dilution water was from the same source of water that supplied the holding tanks. Water in each test vessel was aerated through an air stone.

1.2.2. The insecticide delivery system: A modified Mount and Brungs (1967) proportional dilution apparatus which delivered ten

insecticide concentrations and dilution water control to eleven test vessels was constructed and used in the experiment (Harrison et al. 1975). The mixture between dilution water and insecticide from stock solution produced the highest insecticide concentration and the remaining concentrations were produced by 75 percent dilution of successive concentrations with dilution water. The insecticide delivery apparatus supplied 250 ml of insecticide solution to each vessel at each cycle. The cycle time for each delivery was 4 minutes which provided 95 percent replacement time in 17 hours (Sprague, 1969).

1.2.3 Stock solutions preparation: Prior to each experiment, a fresh stock solution of insecticide was prepared at the desir concentration to provide accurate and sufficient amount of insecticide to the insecticide delivery apparatus during the period of the experiment

1.2.3.1 Acephate stock solution: Technical grade acephate (Orthene, 92 percent soluble powder, Chevron Chemical Company) was dissolved in water (the solubility was approximately 65 percent) to make up the stock solution that provided the highest concentration of acephat to the test vessel at 4,000 mg/L and 75 percent dilution series of successive concentrations at 3,000, 2,250, 1,690, 1,265, 950, 710, 535, 400 and 300 mg/L respectively and also a dilution water to the control test vessel. From preliminary study, this range of concentrations was found to produce 100 percent mortality in the high concentrations and partial mortality and no mortality to fish in the lower concentrations. This range of concentrations was selected to test the effect of acephat on rainbow trout fingerlings in all experiments at different test temperatures.

1.2.3.2. Fenitrothion stock solution: Technical grade fenitrothion (Sumithion, 97 percent emulsifiable concentrate, Sumitomo Chemical Company) was dissolved in propylene glycol to provide fenitrothion to the test vessel at the highest concentration of 10.0 mg/L. By diluting this stock solution with various quantities of dilution water, the diluter delivered fenitrothion to the test vessels in a series of concentrations from 10.0, 7.5, 5.6, 4.2, 3.2, 2.4, 1.8, 1.3, 1.0 and 0.75 mg/L respectively and dilution water to a control test vessel. This range of concentrations was used in all fenitrothion experiments at different test temperatures.

Stock solutions of both insecticides for all experiments were prepared from the same source of technical grade materials using the same method of preparation. Stock solution for each experiment was prepared in the morning prior to beginning the experiment in a glass container and covered with aluminum foil and kept stirred with magnetic stirrer to ensure homogeneous solution. At the beginning of each experiment a proper amount of stock solution was added to each test vessel to make the desired concentrations.

1.3 Experimental procedures

To determine the effects of temperature stress on the acute lethality of acephate and fenitrothion and on ChE inhibition in rainbow trout fingerlings, a series of experiments was designed using the following procedures.

1.3.1. Acute lethality studies. Rainbow trout fingerlings which were acclimated for 3 weeks in the holding tank to 15°C were tested with each insecticide at 3 test temperatures at 8, 15 and 22°C.

Test temperatures at 8°C and 22°C were defined as cold and heat stresses respectively.

Experiments with each insecticide at each test temperature were done in duplicate except the acephate experiment at 15°C, so that a total of 20 fingerlings were tested at each insecticide concentration at each temperature. All data from each insecticide at each temperature were pooled for statistical analysis.

In each experiment, 110 rainbow trout fingerlings of either sex were equally distributed in a random manner into 11 test vessels (10 fish per vessel) containing dilution water at the desired test temperature. Fish were held in the test vessels at the test temperature for 48 hours before the beginning of the experiment. All tests were run for a 96 hour period by starting the test on Monday morning and running continuously until Friday morning. Feeding was withheld after the fish were put in the test vessels and during the experiment.

Treated fish in each test vessel were checked for mortality at half hour intervals for the first six hours and hourly intervals up to twelve hours and at 14, 16, 18, 24, 30, 36, 48, 60, 72, 84 and 96 hours. Criteria for death were the cessation of respiration and lack of response to any tactile stimuli. Dead fish were removed from test vessels and time of death, weight and length of each fish were recorded. Brain and skeletal muscle samples from each fish were excised and frozen using dry ice for ChE analysis. At the end of the 96 hour period, all fish that survived in each test vessel were sacrificed and weight and length were recorded. Brain and skeletal muscle samples were also excised from these fish and frozen for ChE analysis studies.

Water chemistry characteristics of test water in each test vessel

Dissolved oxygen and temperature were measured with a meter (Yellow Spring Instrument Co. Inc., Model 54) and pH with a pH meter (Radiometer Co. Model 29B). Water samples from each test vessel were collected at 48 hour periods for analysis of insecticide concentrations. Analyses were done by the Chemistry Laboratory of the Freshwater Institute, Winnipeg, using gas chromatographic procedures (Grift and Lockhart, 1974; Anon., 1973).

Median lethal concentration (LC_{50}, the concentration of insecticide that produced 50 percent mortality at a definite time period) and confidence intervals were calculated at 24, 48 and 96 hour periods for each experiment by using the probit analysis method of Finney (1971). The prediction of LC_{50} value and its 95 percent confidence interval was based on the conversion of the concentrations tested and the corresponding observed percent mortalities to logs and probits, respectively, and the subsequent mathematical calculation of a linear regression equation. Comparison of the LC_{50} value for each temperature was done by an analysis of variance ($p \leq 0.05$).

Median survival time (MST, time required to produce 50 percent mortality for each concentration of insecticide) and 95 percent confidence intervals for each test were calculated according to the method of Litchfield (1949). A mortality curve for each temperature was constructed between log median survival time (MST) and log concentration; the slope, the y-interception and correlation coefficient (r) were calculated by using regression analysis. Comparison of slope for each mortality curve was done by an analysis of covariance ($p \leq 0.05$) (Snedecor and Cochran, 1967).

Rate of mortality (the reciprocal of the median survival time, 1/MST), a rate expression of toxicity (Macleod and Pessah, 1973; Hodson and Sprague, 1975) for acephate and fenitrothion in each concentration was calculated at each range of temperature changes. Q_{10}, therefore, was calculated to express the ratio of increase of the relative toxicity with an increase in temperature from the formula given by Hoar (1975) as follows:

$$\log Q_{10} = \frac{10(\log k_1 - \log k_2)}{t_1 - t_2}$$

where k_1 and k_2 are rate of reactions which in this case are the rate of mortality, at temperatures t_1 and t_2 respectively.

1.3.2. ChE inhibition in brain and skeletal muscle: Brains from dead fish exposed to insecticide in the acute lethality studies were obtained by cutting off the top of the skull and snipping the brain loose at the optic nerves and base of the medulla. Skeletal muscle samples were obtained from each fish from a lateral section below the dorsal fin. Brain and skeletal muscle samples from control fish and treated survivors were obtained immediately after the 96 hour test period. After weighing, all samples were frozen using dry ice. Frozen brain and muscle samples from each test concentration were placed in individual plastic bags, labelled and stored at -20°C until required for analysis of ChE activities. Experiments using fresh and stored tissues showed that freezing did not significantly affect ChE activity if the analysis was performed within two months (Klaverkamp et al. 1976).

Brain and skeletal muscle samples from dead and surviving fish aft[illegible] exposure at each concentration and temperature were pooled for

analysis of ChE activity. Duplicate analyses were determined for ChE activity for both tissues. Means and standard errors of 4 values obtained from duplicated analyses of pooled samples from 2 replicated experiments were calculated and plotted against insecticide concentrations. ChE activities of brains and skeletal muscles were determined by using a pH stat method with an automatic titrator (Radiometer Co.) coupled to a circulating temperature controlled water-jacket as described by Coppage (1971) and Pickering and Pickering (1971). Homogenates of brains or skeletal muscles were prepared in a physiological saline solution (Wolf, 1963), using a Williams Polytron homogenizer. The millimolar (mM) composition of the modified Cortlands saline solution was: NaCl 139.4, KCl 5.1, $CaCl_2$ 1.2 and $MgSO_4$ 0.9. ChE activities of both brain and skeletal muscle samples were determined at 15°C at end point of pH 8.0 using a final homogenate concentration of 1 mg/ml with 10 mM acetylcholine bromide (AChBr) as the substrate and 0.002 N NaOH as the titrant.

ChE activity was expressed as micro moles of acetylcholine hydrolyzed per milligram of protein per hour (μmoles ACh hydrolyzed/mg protein/hr) and also as percent of control value. Protein determinations of samples for ChE analysis were conducted on aliquots of the homogenates using a modification of the Lowry et al. (1951) method.

2. Cardiovascular/respiratory responses and ChE inhibition

To provide more understanding on the mechanisms and sites of action involved in the toxicity of OP insecticides in fish, studies were conducted on the effects of acephate and fenitrothion on biochemical and physiological parameters of the cardiovascular, respiratory and central nervous systems

2.1 Fish holding and feeding conditions

Fish used in this study were 66 adult rainbow trout (Salmo gairdneri) of either sex with a mean weight of 514 grams (range, 280-750; S.D. ± 125) and a mean length of 34.7 centimeters (range, 30.0 - 39.0; S.D. ±2.5). They were from the same source as the fingerlings used in acute lethality studies. These fish were held in a 522-liter fiber glass holding tank and received a continuous flow of thiosulphate-dechlorinated Winnipeg City tap water (1 to 5 mg/L) at 10°C for at least 1 month prior to use in the study. These fish were fed once daily with dry pellet trout food (EWOS No. 7P, Brood stock food) at the rate of 5.1 gm/day/fish or about 1 percent of body weight. Fish were not fed 24 hours before and during the experiment.

2.2 Experimental conditions

2.2.1 Restraining chambers: 4 plexiglass restraining chambers modified from Smith and Bell (1967) were used. The chambers were constructed of 0.6 centimeter green acrylic sheeting (50 x 15 x 14 centimeters) and each chamber was equipped with movable partitions to restrain the fish. These partitions did not restrict breathing of the fish or movement of the paired fins but prevented swimming in any direction and turning. These chambers were placed in a controlled temperature water bath at 10 ± 1°C. Overflow water ran through inverted Y-tubes at the back of each chamber which kept the volume of water constant at 8 liters. Dissolved oxygen was maintained at near saturation level in each chamber by the use of an air stone. The test chamber and water bath were covered with dark curtains.

2.2.2. Insecticide delivery system: A 4-channel insecticide system modified from Harrison et al. (1975) was constructed. Three test chambers received the same insecticide concentration and the fourth chamber received only dilution water at the flow rate of 0.5 liter per minute which provided 99 percent replacement time of 1.2 hours in each chamber (Sprague, 1969).

The concentration of acephate and fenitrothion selected for testing were 2,000 mg/L and 2.0 mg/L which are approximately the 48 hour LC_{50} values obtained from the acute lethality studies in fingerlings. Stock solution for all experiments for both acephate and fenitrothion were prepared in the same manner as in acute lethality studies.

2.3 Experimental procedures

Experiments were designed to test the effects of each insecticide on rainbow trout at one concentration but different durations of exposures ie. 1, 3, 6, 12, 24 and 48 hours. In each experiment 3 treated fish and a control were tested for each exposure period. The responses of cardiovascular, respiratory, serum electrolyte concentrations and ChE inhibition in various tissues were measured. To obtain additional control data, 2 additional fish exposed to dilution water only were tested, and the responses were measured as the same as the above control fish.

2.3.1. Cardiovascular and respiratory responses: All experiments began between 9 to 10 AM and each fish was anesthetized with 0.33 ml of 2-phenoxyethanol (Sigma Chemical Co.) per liter of dilution water for approximately 10 minutes at 10°C. After anesthetization, the weight and length were measured, and the fish was transferred to an operation table for cannulation and surgical procedures. Anesthetization

was maintained during the surgical procedures by irrigation of the gills with aerated dilution water containing 0.33 ml of 2-phenoxyethanol/L at 10°C.

A buccal cannula consisting of polyethylene tubing (Clay Adams, Intramedic PE 60) was inserted through the snout to obtain buccal respiratory rate and amplitude as described by Saunders (1961). Cycles of buccal pressure change were used as a measure of ventilation rate. Coughing responses were also recognized from the changes in the respiratory trace response (Schaumberg et al. 1967).

To obtain electrocardiogram (ECG) data, a set of 3 electrodes (Hewlett Packard model 14060 K) were used and inserted under the skin of the fish. The first electrode, recording electrode, was inserted midventrally slightly anterior to the pectoral fins and close to the heart. The second electrode, reference electrode, was inserted midventrally at the abdomen just anterior to the pelvic fins. The third electrode, grounding electrode, was placed at the side above the lateral line and below the dorsal fin. All 3 electrodes were sutured in place with polyethylene suture.

Blood samples were obtained from a cannula implanted in the dorsal aorta (Smith and Bell, 1964). The blood catheter (30 centimeters in length) consisting of polyethylene tubing (Clay Adams, Intramedic PE 50) was introduced into the aorta between the fourth gill archs by passing it through a 17 gauge thinwall hypodermic needle. The needle then was removed leaving the cannula inside the blood vessel. Heparinized saline was used in this preparation to prevent blood clotting by mixing modified Cortland saline (Wolf, 1963) with heparin (Sigma Chemical Co.) at 50 units per ml. The dorsal cannula was sewn to the roof of the mouth and passed through the snout.

These procedures, required approximately 15 minutes for each fish after which they were placed in the restraining chamber at 10°C for recovery. Catheters and electrode cables were vented through the chamber top and connected to the recorders.

A physiological recorder (Hewlett Packard model 7754A) was used to record the electrocardiogram (ECG) as well as the respiratory responses. ECG wave forms were obtained by connecting electrodes to a patient cable (HP model 14067 E) which attached to a bioelectric amplifier (HP model 8811 A). The respiratory responses were recorded by attaching the respiratory catheters to a pressure transducer (HP model 1280 C) in series with a pressure amplifier (HP model 8805 C).

Buccal respiratory traces, as well as ECG data were observed immediately after the fish were placed in the chambers. The fish were allowed to recover for 48 hours from the surgical implantation of ECG electrodes and cannulae before they were exposed to an insecticide. Breathing and heart rates and other parameters from each fish were monitored at time intervals from 48 hours until 0 hours before exposure to establish baseline conditions. ECG and respiratory responses of treated and control fish were observed every half hour during the first 6 hours of exposure and hourly until the 12 hour period and then at 24, 36 and 48 hours.

Mean values and 95 percent confidence intervals were calculated for heart rates, respiration rates, buccal amplitudes and coughing rates for each experimental group. For each observation period, the mean values for each group of fish were compared with that of control groups using the Students t-test at the significant level of $p \leq 0.05$.

2.3.2. ChE inhibition in fish tissues: At the end of each experiment blood samples were withdrawn from the dorsal aorta cannulation tubing with a syringe, centrifuged and serum was separated and sealed in glass vacutainers. Red blood cell and serum samples were kept frozen until required for the analysis of ChE activity. ChE analyses of red blood cell and serum were done by the method modified from Aldrich et al. (1969) and Wills (1972).

After blood samples were taken, fish were killed and brain, heart, gill and skeletal muscle samples were also excised from each fish, weighed and stored at -20°C until required for ChE analyses. Brain and skeletal muscle samples were prepared as described in the acute lethality experiment. Heart samples were taken by cutting off the bulbus arteriosus and lifting the heart out from the pericardium. Heart homogenates were prepared in the same manner as the skeletal muscle samples. Gill samples were taken by cutting off the first, second, third and fourth gills and washing them with saline solution. No attempt was made to flush the blood that was left in the gill arches and filaments. Gill homogenates were prepared in the same manner as the other tissues.

The pH stat method as described in the previous experiment was used for all ChE analyses. The ChE analysis conditions are summarized in Table 1. The Lowry et al. (1951) method was used for protein determination of samples. The ChE activity was expressed as micro moles of acetylcholine hydrolyzed per milligram of protein per hour and also as the percent of control value. ChE analyses were done in duplicate. Mean values and standard errors of ChE activity for brain, gill, heart, red blood cell, serum and skeletal muscle were calculated for each experimental group.

Table 1. Summary of cholinesterase analyses conditions in various tissues of rainbow trout.

	Brain	Skeletal Muscle	Heart	Gill	RBC (Red Blood Cell)	Serum
Final Sample Concentration (mg/L)*	2.5	2.5	25	200	-	-
Volume of Sample Used (ml)	1.0	1.0	1.0	1.0	1.0	0.2
Saline Solution Added (ml)	1.0	1.0	1.0	1.0	1.0	1.8
Final Volume (ml)	2.0	2.0	2.0	2.0	2.0	2.0
Substrate Concentration (M)	0.05	0.05	0.05	0.05	0.005	0.10
Volume of Substrate Added (ml)	0.5	0.5	0.5	0.5	0.5	0.2
Final Substrate Concentration (M)	0.01	0.01	0.01	0.01	0.001	0.009
Strength of Titrant Base (NaOH) (N)	0.002	0.002	0.002	0.002	0.001	0.001

NOTES: Enzyme analyses were done by pH stat method, at 10°C, pH end point 8.0 using acetylcholine bromide as the substrate.

* Tissue wet weight

2.3.3. Determination of serum electrolytes: To obtain information on the effects of these OP insecticides on osmoregulation, serum electrolyte concentrations were determined. After blood samples from the dorsal aorta were centrifuged, serum samples were kept frozen until required.

The analyses of Na^{+}, K^{+}, Ca^{++} and Mg^{++} were determined by using an atomic absorption spectrophotometer (Varian Techtron, Model AA5); Cl^{-} was determined by the microtitration method of Schales and Schales (Wolf et al. 1972). All analyses were done by Freshwater Institute Analytical Chemistry Unit, Department of the Environment, Winnipeg. The concentration of serum electrolytes were expressed as milliequivalents per liter (mEq/L) and the mean values and standard errors were calculated for each group of fish. For each exposure period, the mean values for each group of fish were compared with that of control groups using the students t-test at the significant level of $p \leq 0.05$.

RESULTS

1. Effects of temperature on acute lethality and ChE inhibition

1.1. Acute lethality studies

The median lethal concentration values (LC_{50}) and 95 percent confidence intervals for acephate and fenitrothion to rainbow trout fingerlings at 3 test temperatures are presented in Table 2. The 24, 48 and 96 hour LC_{50} values are expressed as milligram of active ingredient of insecticide per liter (mg/L) according to the analytical results. Size of tested fish and physical characteristics of test water are also presented. From these results, it can be seen that the LC_{50} values of acephate to rainbow trout fingerlings are approximately 600 to 1000 times greater than the LC_{50} values of fenitrothion at a given temperature.

Temperature affected the acute lethality of acephate and fenitrothion, especially at the 24 hour period. The 24 hour LC_{50} of 1875 mg/L for acephate at 22°C is significantly different ($p \leq 0.05$) from 3162 and 2893 mg/L at 8° and 15°C respectively. Test temperature did not affect the 48 hour LC_{50} of acephate, which ranged from 1451, 1602 and 1436 mg/L at 8°, 15° and 22°C respectively and the 96 hour LC_{50} which ranged from 724, 852 and 796 mg/L at 8°, 15° and 22°C respectively. In fenitrothion experiments, the 24 hour LC_{50} in all 3 test temperatures are different with a variation from 4.32 mg/L to 2.74 mg/L and 1.94 mg/L at 8°, 15° and 22°C respectively. The 48 hour LC_{50} at 8°C of 2.37 mg/L is also higher than 1.67 and 1.72 mg/L at 15° and 22°C respectively. There is, however, no significant difference ($p \leq 0.05$) for 96 hour LC_{50} values in fenitrothion experiments which range from 1.34, 1.44 and

Table 2. Acute toxicity expressed as LC_{50} values (mg/L) and 95% confidence intervals of acephate and fenitrothion to rainbow trout fingerlings at 3 temperatures.

	Acephate			Fenitrothion		
	8°C	15°C	22°C	8°C	15°C	22°C
24-hour LC_{50}	3162 (2485-4023)	2893 (2146-3899)	1875 (1611-2116)	4.32 (3.32-5.63)	2.74 (2.32-3.25)	1.94 (1.56-2.40)
48-hour LC_{50}	1451 (1236-1704)	1602 (1233-2082)	1436 (1290-1598)	2.37 (1.75-3.21)	1.67 1.42-1.96)	1.72 (1.45-2.04)
96-hour LC_{50}	724 (603-868)	852 (598-1213)	796 (650-974)	1.34 (1.03-1.75)	1.44 (1.19-1.73)	1.39 (1.09-1.77)
Fish Length (cm)±S.D.	7.52±0.28	10.09±1.11	7.67±0.34	9.15±0.87	8.18±0.88	8.63±1.08
Fish Weight (gm)±S.D.	5.96±0.80	12.89±4.60	6.01±0.81	10.56±3.24	7.63±2.60	8.71±3.35
Dissolved Oxygen (mg/L)	7.93±0.30	7.35±0.09	7.51±1.07	11.26±0.17	9.87±0.94	8.42±0.35
pH	6.86±0.61	6.50±0.80	6.98±0.37	7.63±0.08	7.69±0.83	7.78±0.16
Temperature (°C)	8.11±0.01	15.14±0.07	21.92±0.02	8.49±0.16	15.17±0.19	22.21±0.34

1.39 mg/L at 8°, 15° and 22°C, respectively. Figure 2 summarizes the difference in LC_{50} values of acephate and fenitrothion at each time period and temperature.

Median survival times (MST) with 95 percent confidence intervals of fish exposed to acephate and fenitrothion at 3 test temperatures are presented in Table 3. Temperature does not have much affect on the MST,which ranges between 18.28, 23.26 and 17.18 hours at 8°, 15° and 22°C respectively at 4000 mg/L of acephate, the highest concentration tested. The same trend in results, where MST values at 15°C are the highest and MST at 22°C are the lowest, is also observed in lower concentrations of acephate. Temperature showed much more effect on MST values in fish exposed to fenitrothion where MST is decreased as the temperature increases. The MST of rainbow trout exposed to 10.0 mg/L of fenitrothion decreases from 12.13 to 4.06 and 1.22 hours at 8°, 15° and 22°C respectively. The same pattern is also found at other concentrations tested. In general, fish died faster at higher temperatures than at lower temperature of the same concentration of insecticide and this effect was more dramatic in fenitrothion experiments.

Rate of mortality and estimated Q_{10} values for acephate and fenitrothion at each concentration for 2 temperature ranges, 8° to 15° and 15° to 22°C, are presented in Table 4. The estimated Q_{10} values for acephate ranged between 0.68 to 2.02. These values at the temperature range of 8 to 15°C were lower in all cases than at temperature range of 15° to 22°C for the same concentrations. The Q_{10} for fenitrothion ranged between 1.11 to 5.57. These values at the temperature range of

Figure 2. The 24-, 48- and 96-hours median lethal concentrations (LC_{50}) as mg/L and 95 percent confidence intervals of acephate and fenitrothion to rainbow trout fingerlings at 3 temperatures. Numbers within the histogram indicate test temperatures.

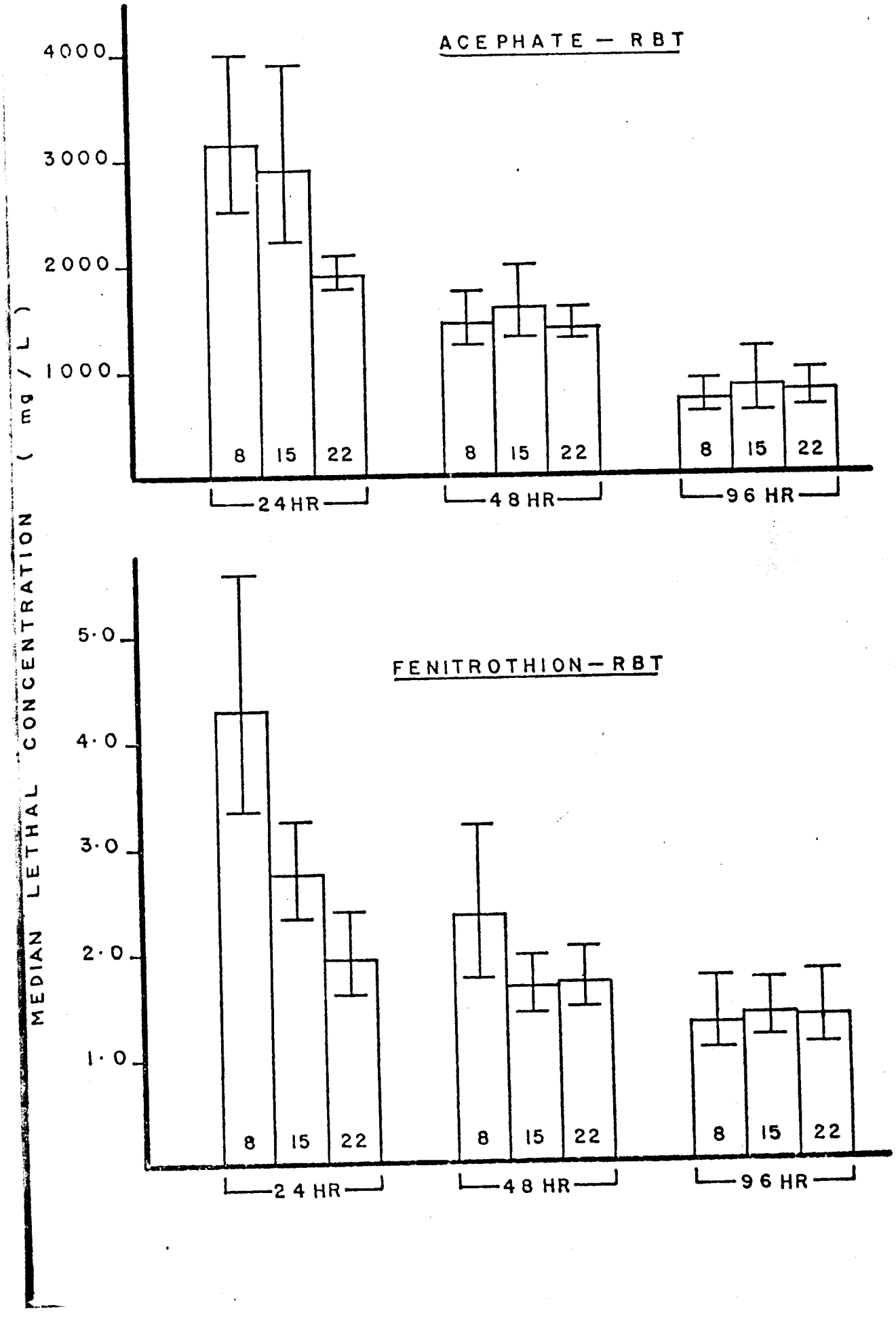

ACEPHATE — RBT
4000
3000
2000
1000
8
15
22
24HR
48HR
96 HR
FENITROTHION—RBT
5·0
4·0
3·0
2·0
1·0
MEDIAN LETHAL CONCENTRATION (mg / L)

Table 3. Median survival time (hours) of rainbow trout fingerlings exposed to acephate and fenitrothion at 3 temperatures.

Median Survival Time (hours) and 95% confidence intervals							
	Acephate				Fenitrothion		
Conc. (mg/L)	8°C	15°C	22°C	Conc. (mg/L)	8°C	15°C	22°C
4,000	18.28 (15.66-21.34)	23.26 (19.14-28.26)	17.18 (13.90-21.23)	10.0	12.13 (10.37-14.19)	4.06 (3.19-5.16)	1.22 (0.95-1.56)
3,000	25.61 (21.70-30.22)	33.54 (27.61-40.75)	22.65 (18.38-27.91)	7.5	17.43 (15.21-19.97)	6.52 (5.22-8.15)	2.70 (2.18-3.40)
2,250	39.48 (32.90-47.38)	45.68 (37.59-55.51)	31.66 (26.38-37.99)	5.6	26.31 (22.68-30.52)	10.35 (8.68-12.33)	5.80 (4.79-7.01)
1,690	55.73 (48.88-63.54)	70.00 (57.62-85.04)	42.68 (34.59-52.67)	4.2	32.95 (29.03-37.40)	18.59 (15.67-22.04)	10.40 (8.53-12.68)
1,265	73.15 (61.99-86.31)	87.97 (84.01-92.11)	65.58 (55.34-77.71)	3.2	48.15 (41.16-56.33)	26.47 (23.12-30.31)	21.25 (17.07-26.46)
				2.4	67.66 (54.12-84.57)	40.76 (34.14-48.66)	38.02 (28.91-50.01)

NOTE: There were no deaths at lower concentrations of acephate and fenitrothion.

Table 4. Rate of mortality (1/MST in hours) and estimated Q10 values for acephate and fenitrothion in each concentration for 2 temperature ranges.

Acephate				Fenitrothion			
Conc. (mg/L)	Temp (°C)	Rate of Mortality	Q10	Conc. (mg/L)	Temp (°C)	Rate of Mortality	Q10
4,000	8	0.0547	0.709	10.0	8	0.0824	4.779
	15	0.0430	1.004		15	0.2463	5.572
	22	0.0582			22	0.8197	
3,000	8	0.0390	0.681	7.5	8	0.0574	4.073
	15	0.0298	1.750		15	0.1534	3.523
	22	0.0441			22	0.3704	
2,250	8	0.0253	0.814	5.6	8	0.0380	3.792
	15	0.0219	1.688		15	0.0966	2.287
	22	0.0316			22	0.1724	
1,690	8	0.0179	0.725	4.2	8	0.0303	2.271
	15	0.0143	2.021		15	0.0538	2.290
	22	0.0234			22	0.0961	
1,265	8	0.0137	0.769	3.2	8	0.0208	2.347
	15	0.0114	1.508		15	0.0378	1.365
	22	0.0152			22	0.0470	
				2.4	8	0.0148	2.054
					15	0.0245	1.106
					22	0.0263	

8^{o} to $15^{o}C$ were higher than Q_{10} values at the temperature range of 15^{o} to $22^{o}C$ except at the highest concentration and at 4.2 mg/L.

Mortality curves for acephate and fenitrothion in rainbow trout fingerlings at 3 test temperatures are constructed using log median survival times and log concentrations (Figure 3 and 4 respectively). Each point represents the median survival time in hours of fish at each concentration and vertical bars represent 95 percent confidence intervals. The regression lines are calculated and constructed through these points and the slopes of each line are determined.

The slopes of acephate mortality curves are not changed significantly ($p \leq 0.05$) with temperature and ranged between -0.94, -0.97 and -1.01 at $8^{o}C$, $15^{o}C$ and $22^{o}C$ respectively. With fenitrothion, the slopes of the mortality curves are changed significantly ($p \leq 0.05$) with increases from -0.88 at $8^{o}C$ to -1.44 at $15^{o}C$ and -2.04 at $22^{o}C$ respectively.

1.2 ChE inhibition in brain and skeletal muscle

1.2.1. Acephate: Cholinesterase activity in brains and skeletal muscles of dead rainbow trout fingerlings after exposure to acephate in the acute lethality studies are presented in Table 5 and ChE activity in brains and skeletal muscles of surviving fish after 96 hours exposure to acephate are presented in Table 6. Brain enzyme activity of dead fish after exposure to acephate varied with concentrations and temperatures tested and ranged from 11 to 24% of control at $8^{o}C$, 17 to 43% at $15^{o}C$ and 12 to 27% at $22^{o}C$. Enzyme activity in skeletal muscle of dead fish ranged from 17 to 38% at $8^{o}C$, 19 to 47% at $15^{o}C$ and 19 to 30% at $22^{o}C$. In general, the brain enzyme activity of dead fish ranged between 10 to 30% of control except at 2 highest concentrations

Figure 3. Mortality curves of acephate to rainbow trout fingerlings at 3 temperatures. Each point represents median survival time in hours. The vertical bars represent 95 percent confidence intervals. The slopes of the best fitted regression line through these points are also presented.

ACEPHATE - RBT

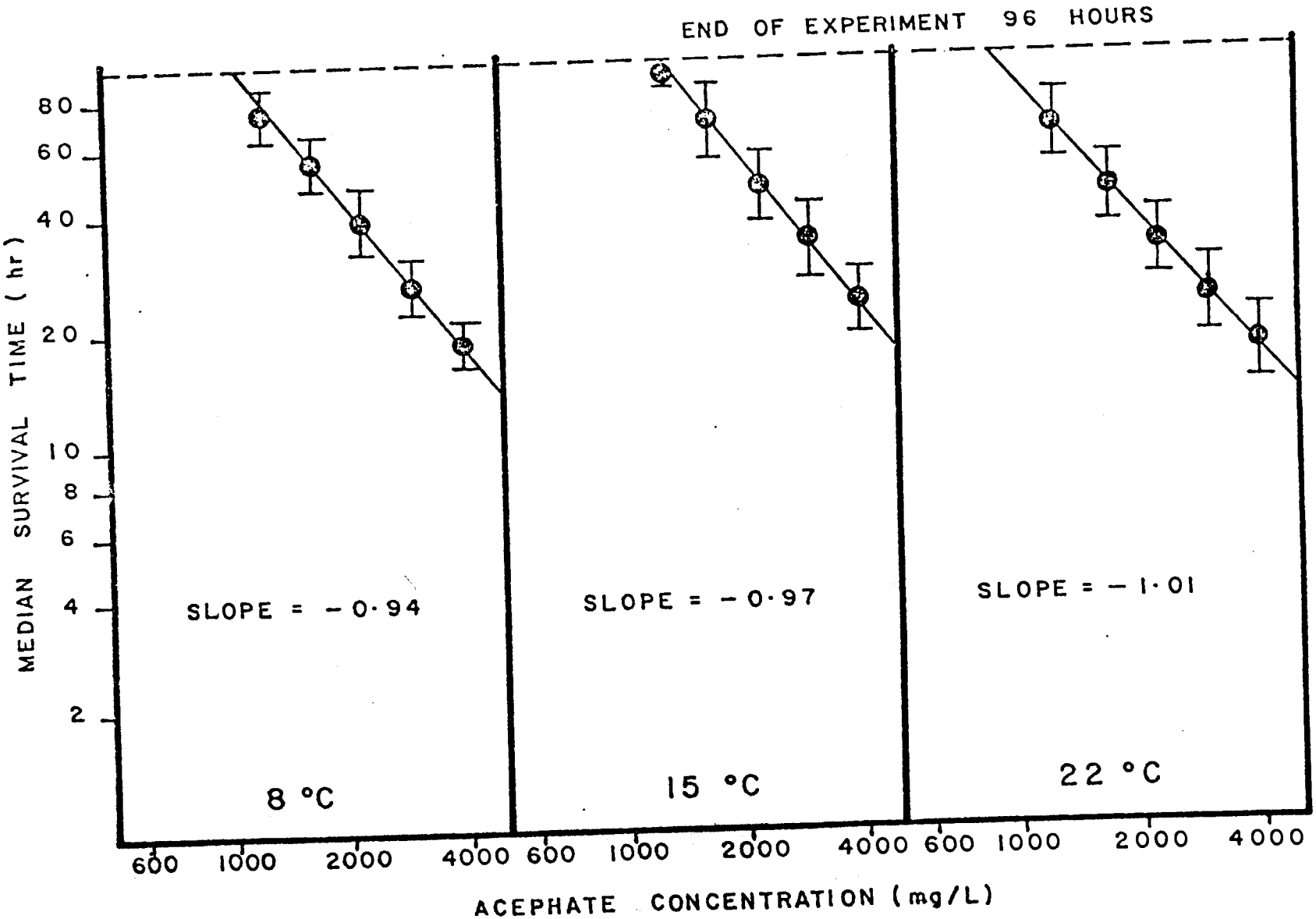


Figure 4. Mortality curves of fenitrothion to rainbow trout fingerlings at 3 temperatures. Each point represents median survival time in hours. The vertical bars represent 95 percent confidence intervals. The slopes of the best fitted regression line through these points are also presented.

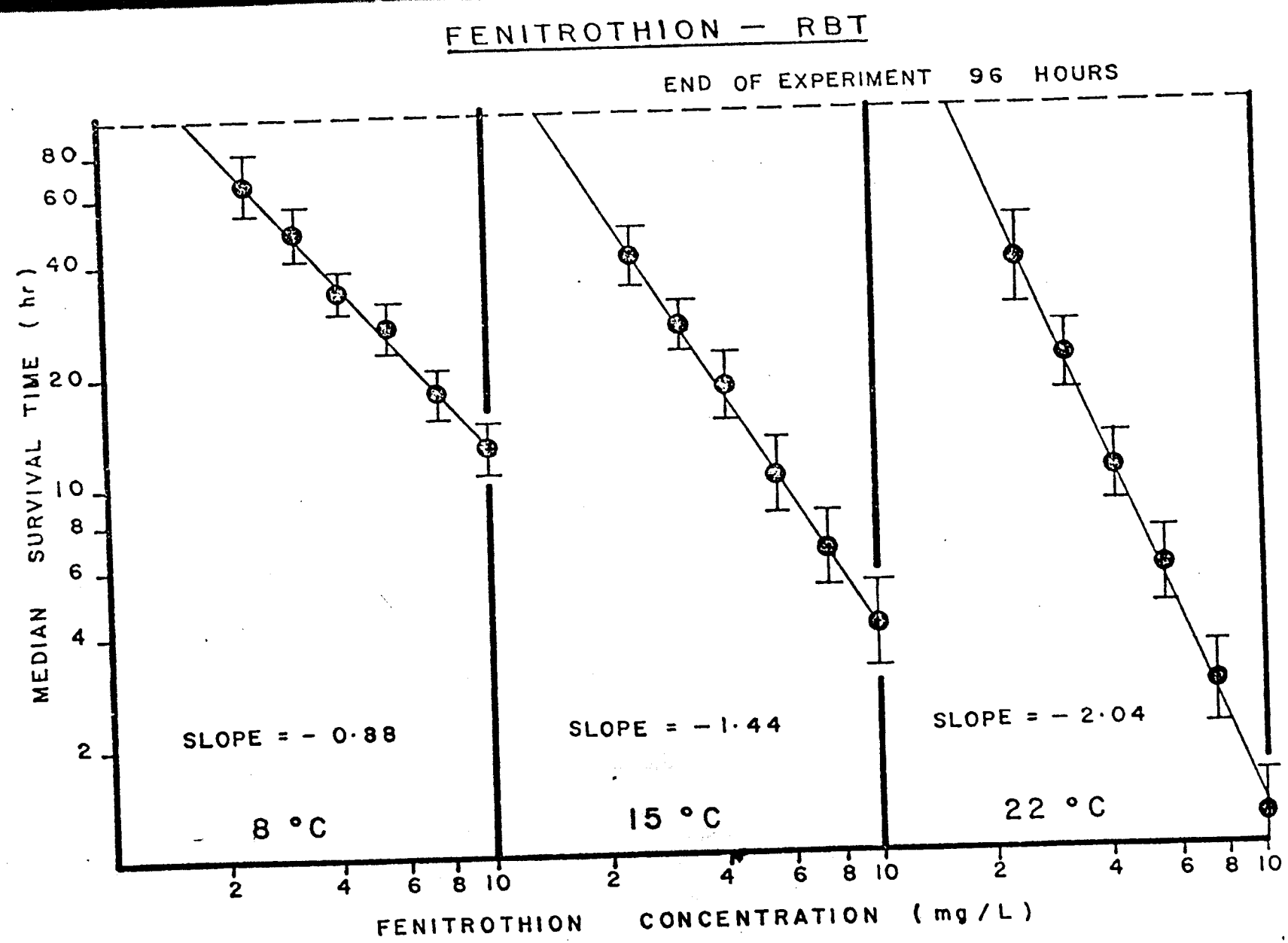

FENITROTHION — RBT
END OF EXPERIMENT 96 HOURS
MEDIAN SURVIVAL TIME (hr)
80
60
40
20
10
8
6
4
2
SLOPE = - 0·88
8 °C
SLOPE = - 1·44
15 °C
SLOPE = - 2·04
22 °C
2
4
6
8
10
FENITROTHION CONCENTRATION (mg / L)

Table 5. Mean ChE activity (μmoles ACh hydrolyzed/mg protein/hr) in brain and skeletal muscle of dead rainbow trout fingerlings after exposure to acephate at 3 temperatures.

Conc.(mg/L)		8°C		15°C		22°C	
		Brain	Skeletal Muscle	Brain	Skeletal Muscle	Brain	Skeletal Muscle
4,000	Mean±S.E.	5.57±1.24	6.53±0.72	8.71±4.57	7.48±5.06	2.90±0.74	2.45±0.14
	Range	4.43-7.12	5.76-7.40	4.75-12.75	3.05-12.16	2.26-3.54	2.29-2.61
	% Control	23.61	37.94	39.30	39.08	17.13	19.05
3.000	Mean±S.E.	4.43±0.18	3.79±0.19	9.44±6.74	9.08±4.26	2.65±0.52	3.83±0.45
	Range	4.18-4.56	3.53-3.98	3.55-15.42	5.19-13.12	1.98-3.26	3.39-4.40
	% Control	18.78	22.02	42.60	47.44	15.65	29.78
2,250	Mean±S.E.	3.97±0.62	4.67±0.60	6.38±2.92	4.86±1.43	2.12±0.20	2.83±0.27
	Range	3.30-4.50	3.98-5.42	3.65-9.15	3.45-6.41	1.94-2.42	2.50-3.05
	% Control	16.83	27.14	28.79	25.39	12.52	22.00
1,690	Mean±S.E.	4.31±0.60	4.06±0.51	4.99±2.91	5.21±1.23	1.98±0.58	2.81±1.07
	Range	3.47-4.87	3.39-4.61	2.41-7.52	4.00-6.36	1.16-2.40	1.88-3.81
	% Control	18.27	23.59	22.52	27.22	11.69	21.85
1,265	Mean±S.E.	2.56±0.61	2.95±1.03	3.84±0.72	3.65±0.27	3.18±0.62	3.05±0.09
	Range	1.69-3.10	1.96-4.00	3.07-4.54	3.38-4.00	2.51-3.71	2.92-3.11
	% Control	10.85	17.14	17.33	19.07	18.78	23.72
950	Mean ±S.E.	4.34±2.61	4.64±1.30	5.51±1.11	4.06±0.21	4.62±1.99	2.89±0.80
	Range	2.15-7.34	3.25-5.75	4.32-6.64	3.78-4.24	2.62-6.41	2.00-3.82
	% Control	18.40	26.96	24.86	21.21	27.29	22.47

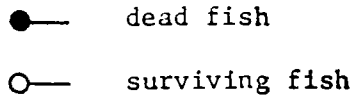
Table 6. Mean ChE activity (μmoles ACh hydrolyzed/mg protein/hr) in brain and skeletal muscle of rainbow trout fingerlings surviving after 96 hours exposure to acephate at 3 temperatures.

		8°C		15°C		22°C	
Conc. (mg/L)		Brain	Skeletal Muscle	Brain	Skeletal Muscle	Brain	Skeletal Muscle
950	Mean±S.E.	3.65±0.31	3.32±0.49	7.24±1.52	4.55±0.57	5.11±0.28	2.87±0.24
	Range	3.20-3.86	2.60-3.72	5.89-8.68	4.00-5.04	4.78-5.46	2.58-3.16
	% Control	15.47	19.29	32.67	23.77	30.18	22.32
710	Mean±S.E.	4.88±1.18	4.01±0.37	7.25±1.80	6.04±0.29	5.21±0.24	3.59±0.52
	Range	3.47-6.12	3.61-4.32	5.62-8.81	5.70-6.32	5.01-5.49	3.12-4.18
	% Control	20.69	23.30	32.72	31.56	30.77	27.92
530	Mean±S.E.	5.70±0.62	4.15±0.39	6.65±1.61	5.70±1.40	5.92±0.40	4.46±0.34
	Range	5.09-6.48	3.68-4.58	5.21-8.17	4.40-6.95	5.57-6.27	4.11-4.83
	% Control	24.16	24.11	30.01	29.78	34.97	34.68
400	Mean ±S.E.	6.45±2.08	5.38±1.41	10.59±0.52	7.77±0.37	6.96±0.79	5.41±0.35
	Range	4.51-8.94	4.18-6.90	10.24-11.36	7.40-8.26	6.17-7.78	5.06-5.83
	% Control	27.34	31.26	47.79	40.59	41.11	42.07
300	Mean±S.E.	8.14±0.82	5.60±0.12	12.20±1.52	10.19±3.25	7.74±0.80	5.90±0.59
	Range	7.20-9.03	5.43-5.69	10.85-13.77	7.33-13.44	7.05-8.55	5.20-6.46
	% Control	34.51	32.54	55.05	53.24	45.69	45.88
0.0 (Control)	Mean±S.E.	23.59±3.89	17.21±5.39	22.16±0.78	19.14±1.27	16.93±2.22	12.86±1.98
	Range	20.23-27.29	12.12-22.70	21.45-23.18	17.90-20.33	14.55-19.52	11.10-14.92

of acephate at 15°C which haye ChE activity at 39 and 43% of control (Table 5). Skeletal muscle enzyme activity of dead fish ranged from 20 to 30% except at the highest concentrations of acephate at 8°C and 2 highest concentrations at 15°C which have activities of 38, 39 and 47% of control, respectively. Fish that survived after 96 hours exposure to acephate exhibited brain enzyme activity ranging from 15 to 35% of control at 8°C, 30 to 55% at 15°C, and 30 to 46% at 22°C, respectively. Skeletal muscle enzyme activity of surviving fish ranged from 19 to 33% of control at 8°C, 24 to 53% at 15°C and 22 to 45% at 22°C. In general, ChE activity in both brain and skeletal muscle of survivals exhibted an increase as the concentration of acephate decreased in all 3 temperatures tested. Figure 5 shows the ChE activity as percent of control in brains and skeletal muscles of dead fish and of those surviving fish after 96 hours exposure to acephate at 3 temperatures.

1.2.2. Fenitrothion: Enzyme activities in brains and skeletal muscles of dead and surviving rainbow trout fingerlings after exposure to fenitrothion are presented in Tables 7 and 8, respectively. The activity of brain ChE activity in dead fish ranged from 15 to 30% of control at 8°C, 24 to 32% at 15°C and 17 to 52% at 22°C. Skeletal muscle enzyme activity of dead fish ranged from 35 to 51% of control at 8°C, 43 to 51% at 15°C and 28 to 84% at 22°C. Surviving fish after 96 hour exposure to fenitrothion at lower concentrations exhibited brain enzyme activity ranging from 23 to 38% of control at 8°C, 21 to 28% at 15°C and 20 to 29% at 22°C. Skeletal muscle enzyme activity ranged from 26 to 51% of control at 8°C, 32 to 51% at 15°C and 15 to 26% at 22°C. The enzyme activities of brain and skeletal muscle of surviving fish after

Figure 5. Cholinesterase activity (percent of control values) in brain (left panels) and skeletal muscle (right panels) of rainbow trout fingerlings dead and those surviving after 96 hours exposure to acephate at 3 temperatures. Each point represents the average of 4 values obtained from duplicated analyses of pooled samples from 2 replicate experiments.



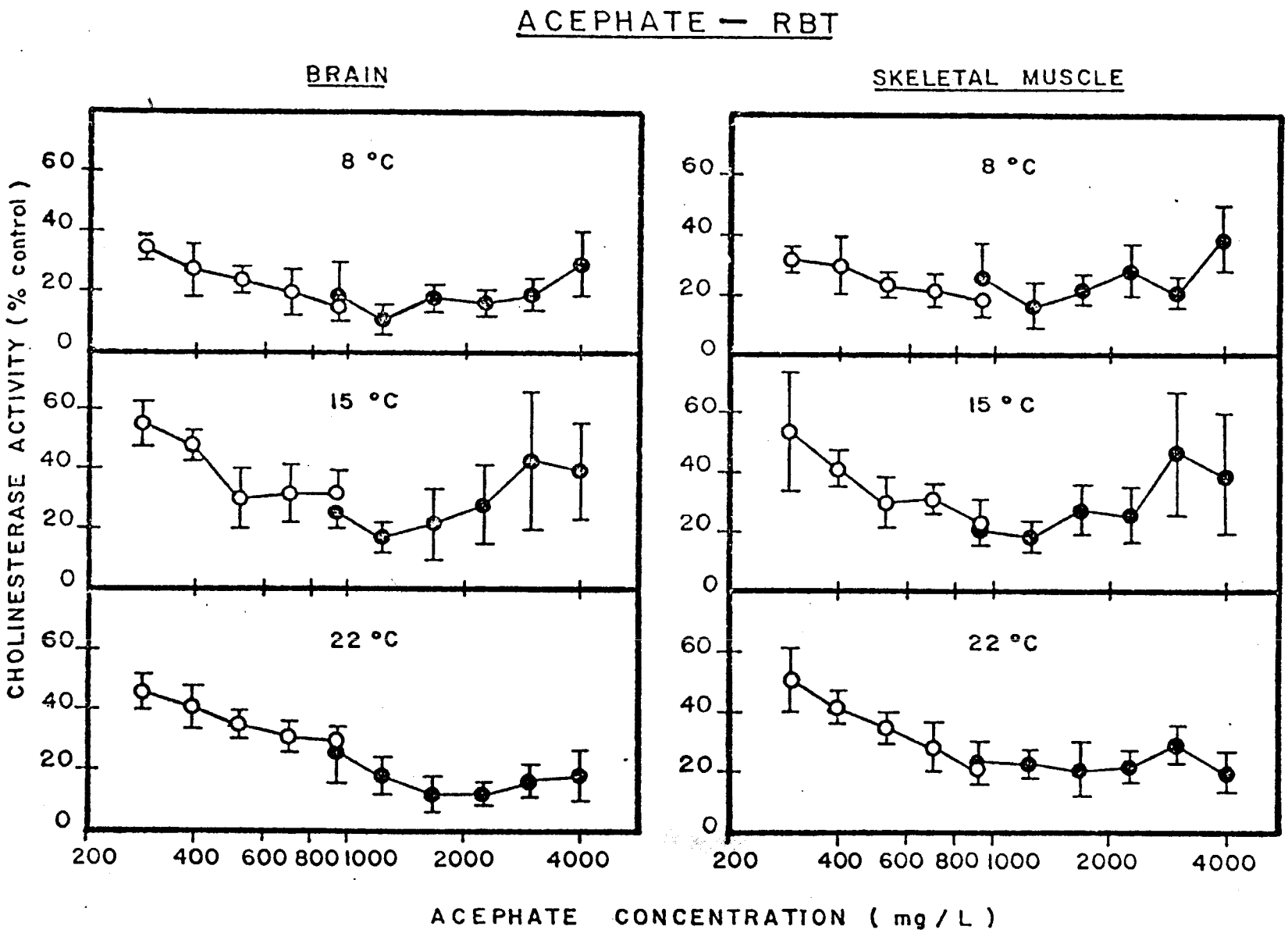

ACEPHATE — RBT
BRAIN
SKELETAL MUSCLE
8 °C
15 °C
22 °C
CHOLINESTERASE ACTIVITY (% control)
0
20
40
60
200
400
600
800
1000
2000
4000
ACEPHATE CONCENTRATION (mg / L)

Table 7. Mean ChE activity (µmoles ACh hydrolyzed/mg protein/hr) in brain and skeletal muscle of dead rainbow trout fingerlings after exposure to fenitrothion at 3 temperatures.

		8°C		15°C		22°C	
Conc. (mg/L)		Brain	Skeletal Muscle	Brain	Skeletal Muscle	Brain	Skeletal Muscle
10.0	Mean±S.E.	3.70±0.34	6.42±2.29	8.81±4.89	6.91±2.28	13.69±5.54	14.47±1.83
	Range	3.41-4.08	4.35-8.45	4.32-13.23	4.97-9.36	8.46-18.60	12.10-16.24
	% Control	14.59	36.15	32.06	43.93	52.29	83.64
7.5	Mean±S.E.	4.88±1.59	6.69±3.29	7.97±4.71	6.98±2.39	10.04±3.67	8.51±1.63
	Range	3.42-6.31	3.75-9.60	3.76-12.32	4.62-9.16	6.86-14.13	6.87-10.17
	% Control	19.24	37.67	29.00	44.37	38.39	49.19
5.6	Mean±S.E.	4.69±1.11	6.29±0.91	7.97±3.63	7.95±2.03	9.18±1.44	8.82±1.23
	Range	3.40-5.62	5.44-7.14	4.70-11.22	6.08-10.15	7.93-10.57	7.42-9.97
	% Control	18.49	35.42	29.00	50.54	35.06	50.98
4.2	Mean±S.E.	5.65±1.44	8.10±2.54	6.84±1.01	6.92±1.18	5.16±0.99	6.72±1.22
	Range	4.41-7.01	5.79-10.39	5.67-7.83	5.55-8.02	4.06-6.46	5.45-7.84
	% Control	22.28	45.61	24.89	43.99	19.71	38.84
3.2	Mean±S.E.	6.56±2.17	8.81±3.57	7.32±1.15	6.99±0.80	6.54±2.92	6.89±3.15
	Range	4.68-8.61	5.81-11.93	6.05-8.59	6.15-7.86	4.01-9.27	4.11-9.65
	% Control	25.87	49.60	26.64	44.44	24.98	39.83
2.4	Mean±S.E.	7.25±2.05	9.09±3.29	7.97±1.48	6.81±0.50	6.91±1.12	8.81±1.60
	Range	5.48-9.16	6.10-12.00	6.85-10.16	6.41-7.52	5.54-8.19	7.15-10.91
	% Control	28.59	51.18	29.00	43.29	26.39	50.92
1.8	Mean±S.E.	7.71±2.26	8.50±2.48	6.68±1.81	7.02±0.21	4.51±0.36	4.87±1.18
	Range	5.59-9.80	6.31-10.68	5.24-9.19	6.74-7.20	4.06-4.94	3.31-5.90
	% Control	30.40	47.86	24.31	44.63	17.23	28.15

Table 8. Mean ChE activity (μmoles ACh hydrolyzed/mg protein/hr) in brain and skeletal muscle of rainbow trout fingerlings surviving after 96 hours exposure to fenitrothion at 3 temperatures.

Conc.(mg/L		8°C		15°C		22°C	
		Brain	Skeletal Muscle	Brain	Skeletal Muscle	Brain	Skeletal Muscle
1.8	Mean±S.E.	5.96±2.66	4.55±1.97	5.65±2.63	5.07±1.05	5.30±2.31	2.60±0.97
	Range	3.03-8.43	2.80-6.34	2.77-8.00	3.72-6.18	3.17-7.65	1.67-3.56
	% Control	23.50	25.62	20.56	32.23	20.24	15.03
1.3	Mean±S.E.	7.60±2.17	6.13±0.62	7.53±1.21	7.37±1.16	6.61±2.33	4.42±0.33
	Range	5.49-9.53	5.45-6.73	6.07-8.56	5.89-8.38	4.47-9.04	4.13-4.89
	% Control	29.97	34.51	27.40	46.85	25.25	25.55
1.0	Mean±S.E.	9.62±2.42	7.30±0.87	7.13±1.87	7.48±1.01	7.51±3.38	4.54±0.31
	Range	7.37-11.87	6.39-8.13	4.91-9.02	6.28-8.47	4.37-10.63	4.37-5.01
	% Control	37.93	41.10	25.95	47.55	28.69	26.24
0.7	Mean±S.E.	9.48±2.55	9.12±2.53	7.70±1.22	7.96±0.72	7.49±2.02	4.36±1.36
	Range	6.98-11.68	6.88-11.37	6.56-9.04	7.04-8.65	5.74-9.33	2.90-5.79
	% Control	37.38	51.35	28.02	50.60	28.61	25.20
0.0 (Control)	Mean±S.E.	25.36±1.60	17.76±5.45	27.48±1.96	15.73±1.51	26.18±0.92	17.30±1.69
	Range	23.78-26.46	12.70-22.53	25.25-29.42	13.67-17.24	25.30-27.47	15.20-19.11

exposure to fenitrothion are generally in the same range as the activity in dead fish. Figure 6 shows the ChE activity (as % of control) in brain and skeletal muscle of dead and surviving fish after exposure to fenitrothion at 3 temperatures.

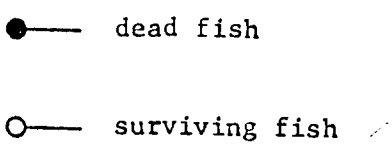
In summary, both acephate and fenitrothion produced ChE inhibition in brain and skeletal muscle in rainbow trout fingerlings in all concentrations tested. Brain enzyme in rainbow trout fingerlings has higher specific activity than skeletal enzyme and is inhibited by each insecticide to a greater extent than skeletal muscle enzyme (Tables 6 and 8). However, the levels of enzyme activity in dead and surviving fish in both brain and skeletal muscle are not significantly different, and it is not possible to distinguish a critical level of enzyme activity which would indicate death.

2. Cardiovascular/respiratory responses and ChE inhibition

2.1. Cardiovascular and respiratory responses

2.1.1. Acephate: Heart rates, respiration rates, buccal amplitude and cough frequency of adult rainbow trout exposed to acephate at 2,000 mg/L are summarized in Table 9. No data were obtained for 48 hour period since all fish died after 24 hours of exposure. The average heart rates of control and treated fish before exposure to acephate are quite stable and range from 54 to 57 beats/min. When acephate was introduced, the heart rates of treated fish were significantly ($p \leq 0.05$) slower than control at 32 beats/min at 1 hour, recovering to 36 and 42 beats/min at 3 and 6 hours after exposure, respectively, but decreasing again to 36 and 35 beats/min at 12 and 24 hours, respectively. Respiration rates of acephate-treated fish increased significantly ($p \leq 0.05$) from 76 beats/min before treatment to 87 and 88 beats/min at 1 and 3 hours after

Figure 6. Cholinesterase activity (percent of control values) in brain (left panels) and skeletal muscle (right panels) of rainbow trout fingerlings dead and those surviving after 96 hours exposure to fenitrothion at 3 temperatures. Each point represents the average of 4 values obtained from duplicated analyses of pooled samples from 2 replicate experiments.



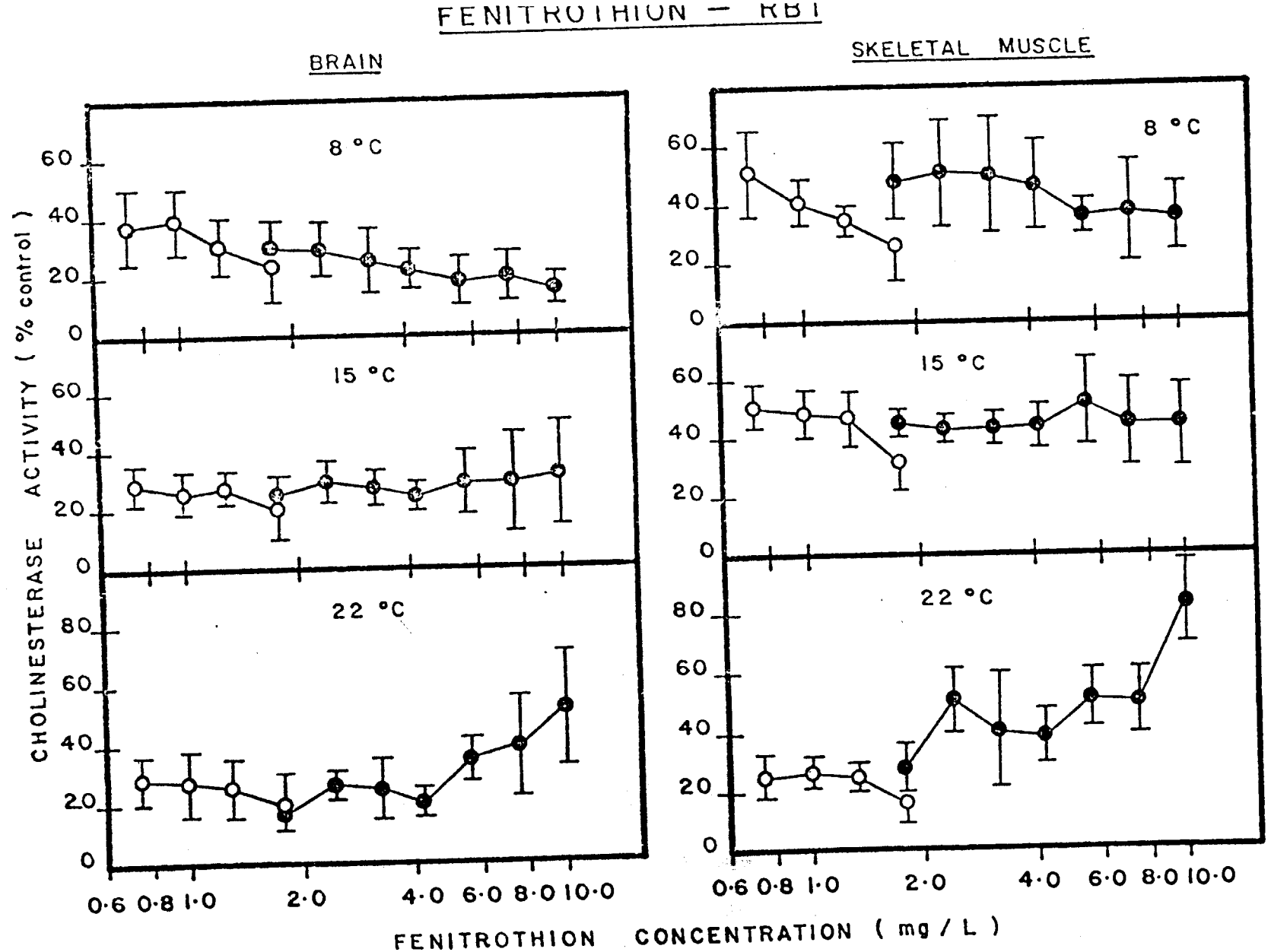

FENITROTHION — RBT
BRAIN
SKELETAL MUSCLE
8 °C
15 °C
22 °C
CHOLINESTERASE ACTIVITY (% control)
0 20 40 60
0 20 40 60
0 20 40 60 80
0·6 0·8 1·0 2·0 4·0 6·0 8·0 10·0
FENITROTHION CONCENTRATION (mg / L)

Table 9. Mean and 95% confidence limits of heart rates (beats/min), respiration rates (beats/min), buccal amplitudes (mm Hg) and cough frequencies (coughs/min) of rainbow trout exposed to acephate at 2000 mg/L.

Exposure Time (hr)	Heart Rates		Respiration Rates		Buccal Amplitudes		Cough Frequencies	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
-24 hr	57.0 (51.5-63.1)	56.7 (55.7-57.6)	79.2 (76.0-82.4)	80.2 (78.9-81.4)	1.45 (1.34-1.57)	1.46 (1.39-1.53)	0.88 (0.67-1.16)	0.92 (0.79-1.07)
-12 hr	56.5 (53.6-59.6)	55.5 54.3-56.7)	77.3 74.4-80.3)	76.2 75.5-76.9)	1.35 (1.26-1.46)	1.40 (1.35-1.45)	1.19 (0.99-1.43)	1.16 (0.99-1.36)
0 hr	54.8 (51.5-58.3)	54.2 (53.0-55.5)	76.7 (74.5-79.0)	76.8 (75.7-77.9)	1.37 (1.28-1.47)	1.31 (1.27-1.36)	1.06 (0.89-1.25)	1.01 (0.88-1.16)
+1 hr	55.8 (53.6-58.1)	32.1 (25.1-41.1)	77.3 (74.9-79.6)	86.7 (81.4-92.4)	1.34 (1.19-1.49)	2.73 (2.12-3.52)	1.34 (1.04-1.70)	1.06 (0.84-1.34)
+3 hr	56.7 (54.6-58.8)	36.0 (27.4-47.2)	78.7 (77.4-80.7)	87.8 (81.9-94.1)	1.44 (1.31-1.58)	2.46 (1.66-3.64)	1.34 (0.99-1.81)	0.99 (0.79-1.23)
+6 hr	54.9 (51.4-58.5)	42.2 (34.2-52.2)	77.8 (74.2-81.6)	86.6 (77.9-96.4)	1.41 (1.28-1.56)	2.33 (1.56-3.49)	0.92 (0.62-1.36)	1.19 (0.87-1.64)
+12 hr	54.6 (50.6-58.9)	36.4 (28.6-46.2)	78.8 (77.1-80.6)	86.5 (74.2-100.8)	1.37 (1.34-1.39)	2.23 (1.33-3.73)	1.03 (0.65-1.64)	1.40 (1.11-1.76)
+24 hr	55.9 (55.7-56.2)	35.8 (29.5-43.3)	79.1 (76.7-81.5)	89.3 (75.8-102.8)	1.36 (1.32-1.41)	1.85 (0.41-3.35)	1.18 (0.58-2.39)	0.95 (0.46-1.95)

exposure. These rates remained elevated at the range of 86 to 89 beats/min compared to 76 to 80 beats/min in control fish. Acephate also caused a significant ($p \leq 0.05$) increase in buccal amplitude from 1.3 mm Hg before treatment to 2.7 and 2.5 mm Hg at 1 and 3 hours after exposure but decreased slightly to the range of 1.8 to 2.3 mm Hg compared to control fish which range between 1.3 to 1.5 mm Hg. Cough frequency in treated fish was not affected by acephate which varied in the range between 0.9 to 1.4 coughs/min as compared to 0.9 to 1.3 coughs/min range in control fish. Figure 7 shows the effects of acephate on heart rates, respiration rates, buccal amplitude and cough frequency responses of treated fish compared to control fish at each exposure time. In summary, acephate produces a decrease in heart rate, an increase in respiration rate and buccal amplitude, especially during the first 3 hours of exposure, and no change in cough frequency.

2.1.2. Fenitrothion: Table 10 summarizes the effects of fenitrothion at 2.0 mg/L on heart rate, buccal amplitude and cough frequency on rainbow trout. Fenitrothion produced a significant ($p \leq 0.05$) decrease in heart rate of treated fish at the first 6 hours after exposure with a range from 43 to 47 beats/min compared to control fish which range between 51 and 52 beats/min. Respiration rates are significantly ($p \leq 0.05$) increased to the range of 84 to 90 beats/min compared to control fish which range between 68 to 70 beats/min. Respiration rates of fenitrothion-treated fish return to near normal after 24 hours of exposure. Buccal amplitude of fenitrothion-treated fish are higher but not significantly different ($p \leq 0.05$) from control fish and ranged from 1.0 to 1.5 mm Hg range in control fish. Cough frequency

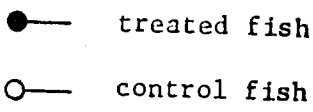


Figure 7. Effects of acephate at 2000 mg/L on heart rates, respiration rates, buccal amplitudes and cough frequency responses in adult rainbow trout. Each point represents the mean and vertical lines represent 95 percent confidence intervals from at least 3 fish.

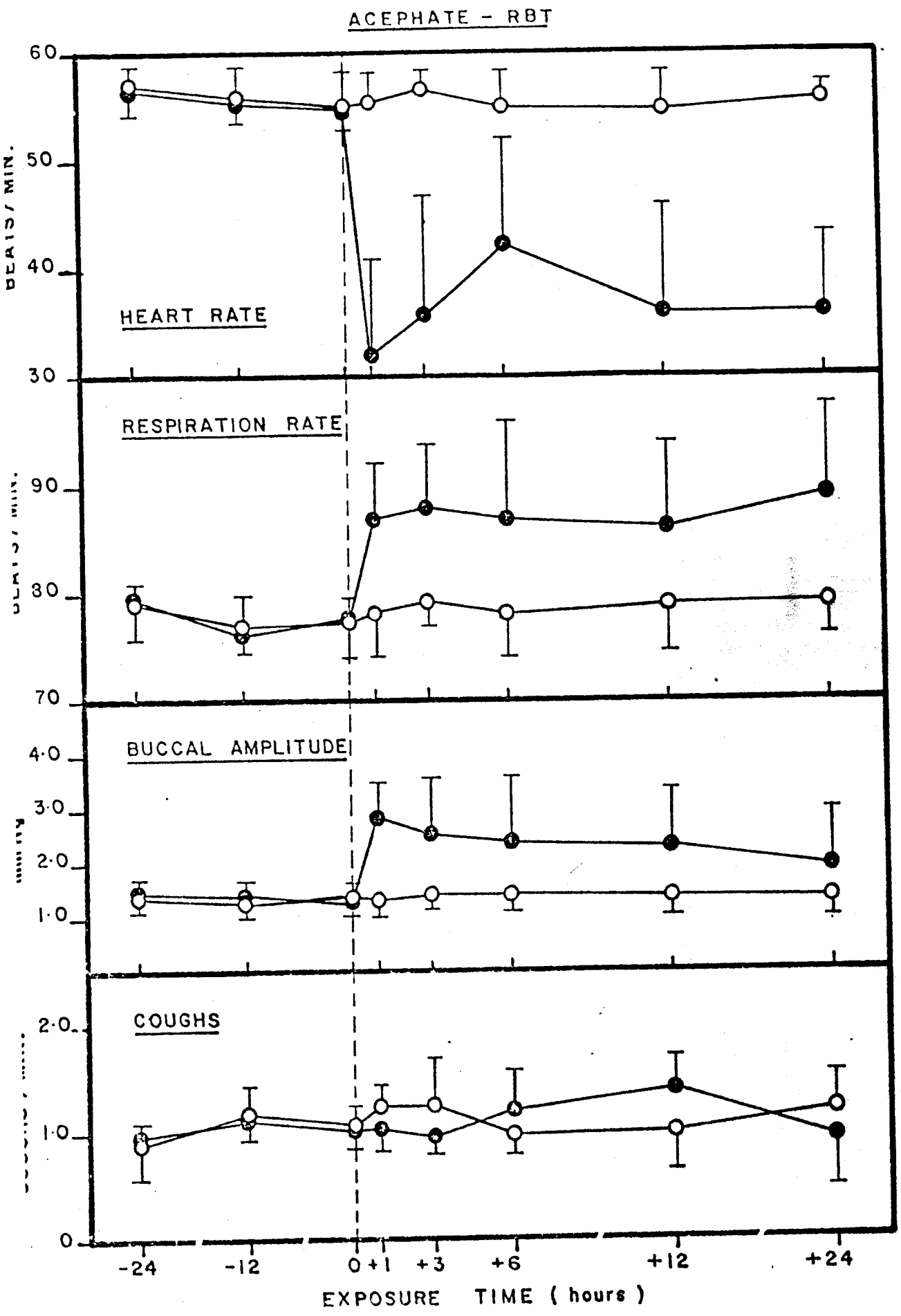

ACEPHATE - RBT
60
50
40
30
BEATS / MIN.
HEART RATE
90
80
70
RESPIRATION RATE
4·0
3·0
2·0
1·0
BUCCAL AMPLITUDE
2·0
1·0
0
COUGHS
-24
-12
0
+1
+3
+6
+12
+24
EXPOSURE TIME (hours)

Table 10. Mean and 95% confidence limits of heart rates (beats/min), respiration rates (beats/min), buccal amplitudes (mm Hg) and cough frequencies (coughs/min) of rainbow trout exposed to fenitrothion at 2.0 mg/L.

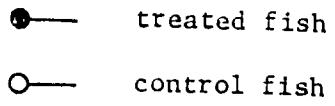
Exposure Time (hr)	Heart Rates		Respiration Rates		Buccal Amplitude		Cough Frequencies	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
-24 hr	51.6 (49.9-53.3)	51.1 (50.2-52.0)	69.6 (67.1-72.2)	69.6 (67.4-71.8)	1.06 (0.99-1.13)	1.13 (1.04-1.23)	1.04 (0.83-1.31)	0.87 (0.76-0.99)
-12 hr	51.6 (50.4-52.8)	52.2 (51.5-52.9)	68.1 (65.2-71.1)	69.1 (68.1-70.1)	1.08 (1.05-1.12)	1.06 (1.01-1.12)	0.76 (0.68-0.86)	0.84 (0.78-0.90)
0 hr	50.7 (49.9-51.5)	50.8 (49.8-51.8)	69.3 (68.2-70.4)	68.3 (66.5-70.2)	1.12 (1.06-1.17)	1.05 (0.99-1.12)	0.86 (0.71-1.05)	0.94 (0.85-1.05)
+ 1 hr	50.9 (49.7-52.2)	43.8 (40.5-47.3)	68.8 (68.2-69.5)	84.9 (78.3-91.9)	1.08 (0.98-1.19)	1.24 (1.06-1.44)	0.77 (0.59-1.01)	6.46 (4.99-8.37)
+ 3 hr	51.4 (49.8-53.1)	46.4 (43.8-49.3)	69.8 (68.0-71.6)	89.9 (81.6-99.1)	1.15 (1.05-1.26)	1.37 (1.12-1.69)	0.85 (0.58-1.24)	8.36 (6.22-11.24)
+ 6 hr	51.6 (49.5-53.7)	44.6 (40.4-49.2)	69.9 (68.6-71.3)	89.3 (78.3-101.8)	1.09 (0.99-1.21)	1.52 (1.12-2.06)	0.99 (0.63-1.55)	7.80 (5.44-11.18)
+12 hr	51.3 (49.4-53.2)	43.1 (34.4-53.9)	68.2 (65.5-71.1)	87.5 (77.6-98.6)	1.08 (1.06-1.09)	1.28 1.00-1.64)	1.17 (0.74-1.87)	9.62 (6.40-14.48)
+24 hr	51.6 (47.5-56.1)	47.6 (42.0-53.9)	69.4 (66.0-72.9)	75.1 (68.2-82.6)	1.12 (1.08-1.17)	1.30 (0.94-1.79)	1.13 (0.87-1.46)	10.88 (6.20-19.09)
+48 hr	51.8 (50.9-52.9)	46.9 (38.6-56.9)	69.2 (68.8-69.6)	74.6 (56.8-97.8)	1.09 (1.08-1.10)	1.19 (0.81-1.72)	0.76 (0.58-0.99)	9.89 (6.46-16.95)

of fenitrothion-treated fish is significantly ($p \leq 0.05$) increased from 0.7 to 1.2 coughs/min range in control and range between 6.4 to 10.9 coughs/min. In summary, fenitrothion produces a decrease in heart rate, an increase in respiration rate and buccal amplitude in rainbow trout as observed in the acephate study, but also caused an increase in cough frequency which was not found in acephate experiments. Figure 8 shows the effect of fenitrothion on heart rate, respiration rate, buccal amplitude and cough frequency of rainbow trout at different times of exposure.

2.2 Electrical activity of the heart

Figures 9 and 10, 11 and 12 show examples of electrocardiogram (ECG) changes recorded from rainbow trout during exposure to acephate and fenitrothion. From ECG configurations (Satchell, 1971) of fish at 0 hour before treatment) in each figure, it can be seen that three main waves, the P-wave, QRS complex and the T-wave of mammalian systems are also present in fish. The P-wave is the first wave in an ECG cycle and corresponds to electrical activity created by myocardial depolarization of the atrium. The QRS complex is the wave of electrical activity resulting from depolarization of the ventricle and repolarization of the atrium. The beginning of this wave complex start about 0.2 seconds after the start of the P-wave. Repolarization of the ventricle gives rise to the last wave of ECG, the T-wave. The T-wave began approximately 0.4 seconds after the start of the QRS complex. No attempt was made in this study to measure the amplitude (voltage) of the ECG waves since the placement of ECG electrodes within the fish has a large influence on the amplitude of ECG. If the electrodes were placed deeper into the surrounding muscle ventral to the heart, the waves were much larger than if placed

Figure 8. Effects of fenitrothion at 2.0 mg/L on heart rates, respiration rates, buccal amplitudes and cough frequency responses in adult rainbow trout. Each point represents the mean of at least 3 fish and vertical lines represent 95 percent confidence intervals.



FENITROTHION – RBT

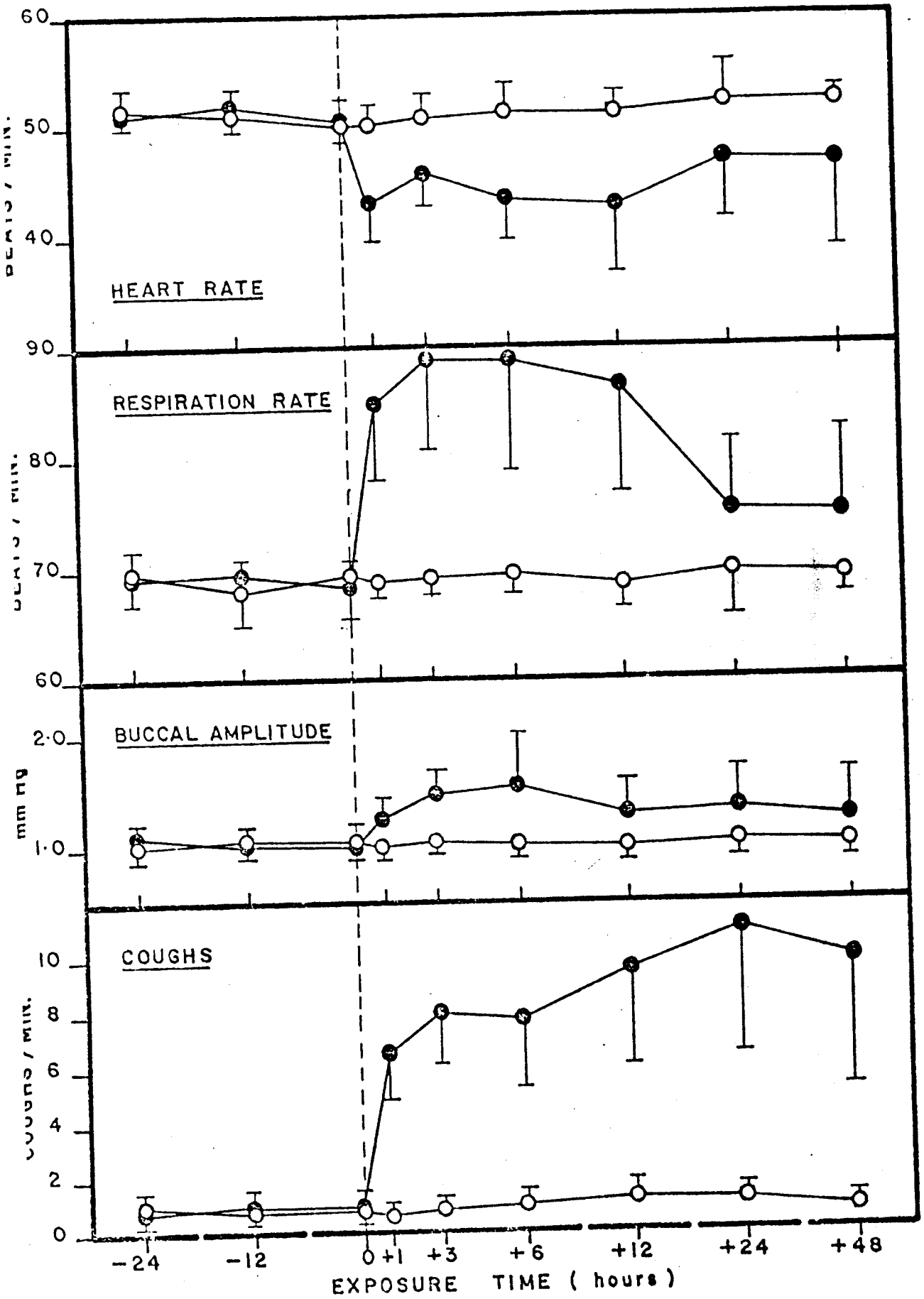


Figure 9. Examples of electrocardiogram (ECG) changes in adult rainbow trout during exposure to acephate at 2000 mg/L demonstrating:

A. Normal ECG pattern of fish at 0 hr, (before acephate was introduced).

B. ECG after 1 hr exposure, showing the changes in pattern of waveforms and duration, P-wave and T-wave become larger and with prominent U-wave (see text).

C,D,E and F. Shows ECG of fish after 3, 6, 9 and 12 hr exposure respectively, showing the increase in magnitude of the T-wave.

G. ECG of fish after 24 hr exposure, showing the change of QRS complex and T-wave still large.

ACEPHATE – RBT

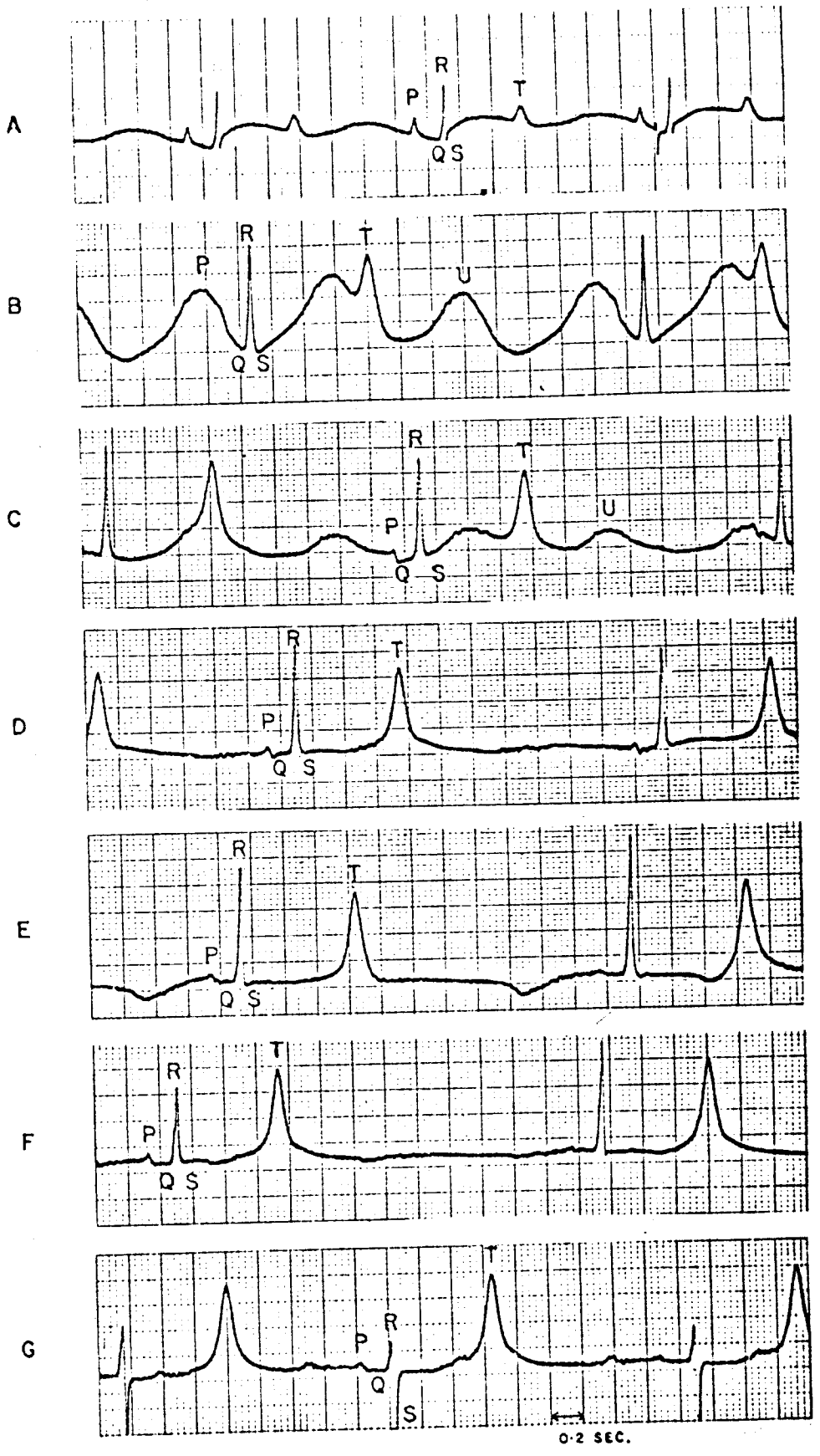


Figure 10. Examples of electrocardiogram (ECG) changes in adult rainbow trout during exposure to acephate at 2000 mg/L demonstrating:

A. ECG at 0 hr.

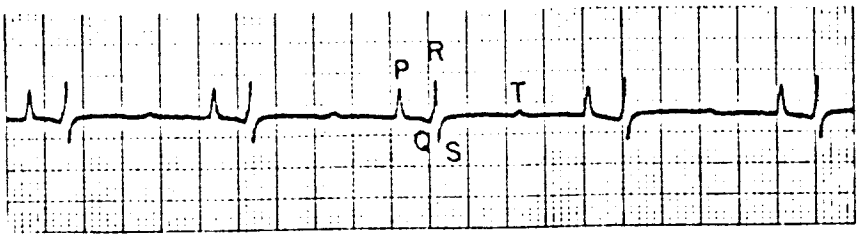
B. ECG after 1 hr exposure, showing missed heart beats.

C. ECG after 3 hr exposure, showing irregular heart beats and changes in QRS complex.

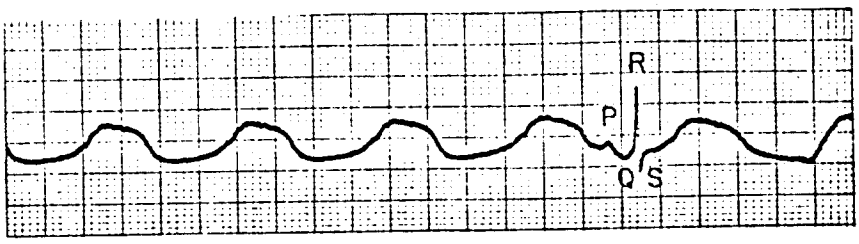
D and E. ECG after 6 and 12 hr exposure showing changes in QRS complex, inverse T-waves and appearance of U-waves.

ACEPHATE - RBT

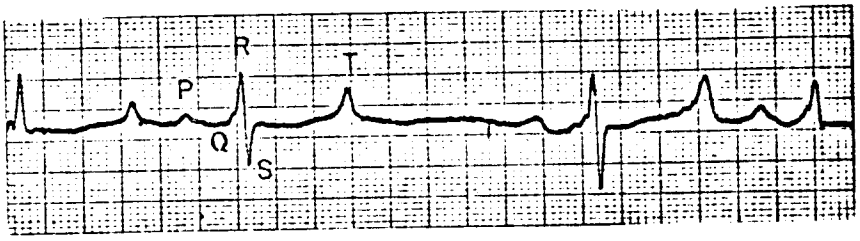
A



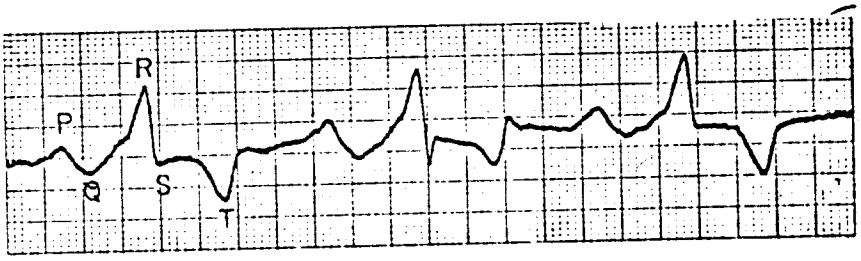
B



C



D



E

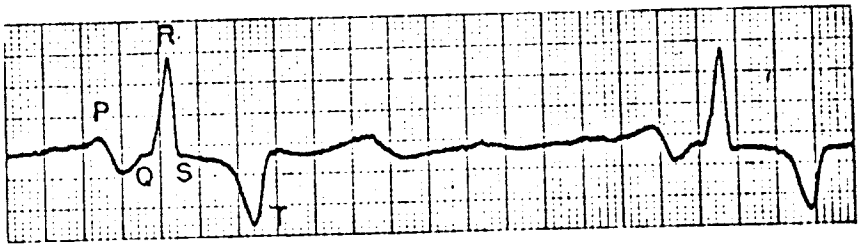


Figure 11. Examples of electrocardiogram (ECG) changes in adult rainbow trout during exposure to fenitrothion at 2.0 mg/L demonstrating:

A. ECG at 0 hr.

B and C. ECG after 1 and 3 hr exposure, showing changes in the waveforms and magnitude.

D. ECG after 6 hr exposure, showing recovery of ECG and irregular waveforms.

E and F. ECG after 12 and 24 hr exposure, showing changes in magnitude of QRS complex and inverse T-wave.

FENITROTHION – RBT

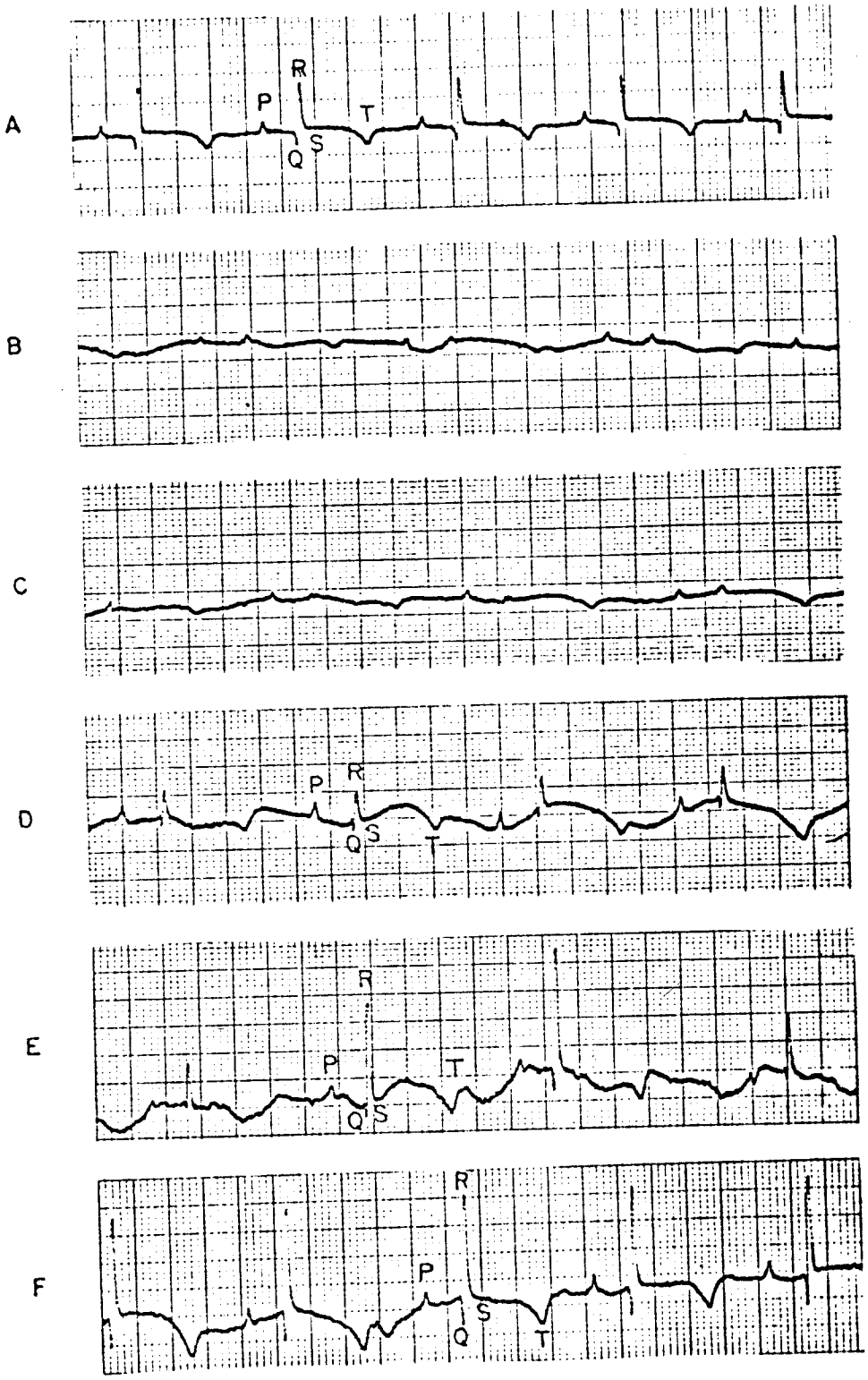


Figure 12. Examples of elctrocardiogram (ECG) changes in adult rainbow trout during exposure to fenitrothion at 2.0 mg/L demonstrating:

A. ECG at 0 hr.

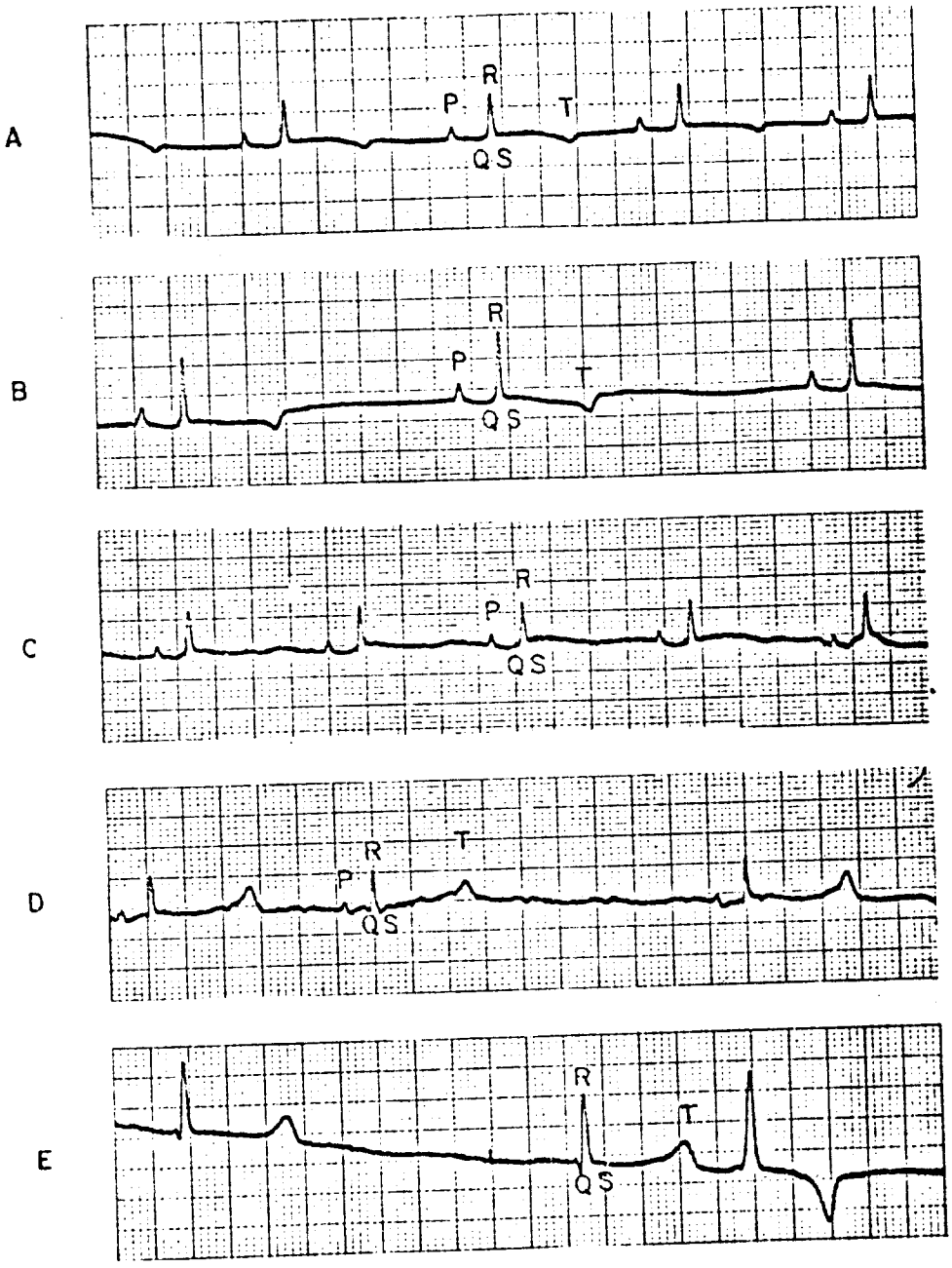
B. ECG after 1 hr exposure, showing changes in duration of ECG cycle.

C. ECG after 3 hr exposure, showing the recovery of ECG.

D. ECG after 6 hr exposure, showing the irregular duration of ECG cycle and enlarged T-wave.

D. ECG after 12 hr exposure, showing the irregular waveform with large T-wave.

FENITROTHION – RBT



just under the skin. The measurement of wave intervals of ECG in treated fish were also very difficult to measure in some cases because the end of each wave especially the T-wave was not well defined. The diagnosis of ECG patterns in treated fish depended upon the changes in the direction and amplitude of each wave form compared to the ECG patterns before treatment of the same fish.

The cardiac electrical activities of fish exposed to acephate and fenitrothion both exhibited changes in frequency and conduction time, as measured by intervals between waves, in the same manner. They showed an initial slowing of the normal rhythm followed by changes in the wave forms. The T-waves changed during exposure period by becoming progressively larger both in amplitude and duration. In some fish, the T-wave changed to a very large upright wave but some fish exhibited an inverse T-wave or depression of the ST segment. Another major change observed in ECG recording was the QRS complex which changed in amplitude, wave form and duration. In some fish, a prominent deflection, called the U-wave, followed the T-wave and preceded the next P-wave. The exact cause of the U-wave is unknown but is currently throught to be the result of the slow repolarization of the intraventricular (Purkinje) conduction system (Goldman, 1973).

In summary, the major characteristics of changes in ECG wave forms in fish during exposure to acephate and fenitrothion are the increase in magnitude of the T-wave with the appearance of tall and slender T-wave, prolongation of the QRS duration and changes in amplitude, transitory ST segment deviation and T-wave inversion.

2.3 ChE inhibition in fish tissues

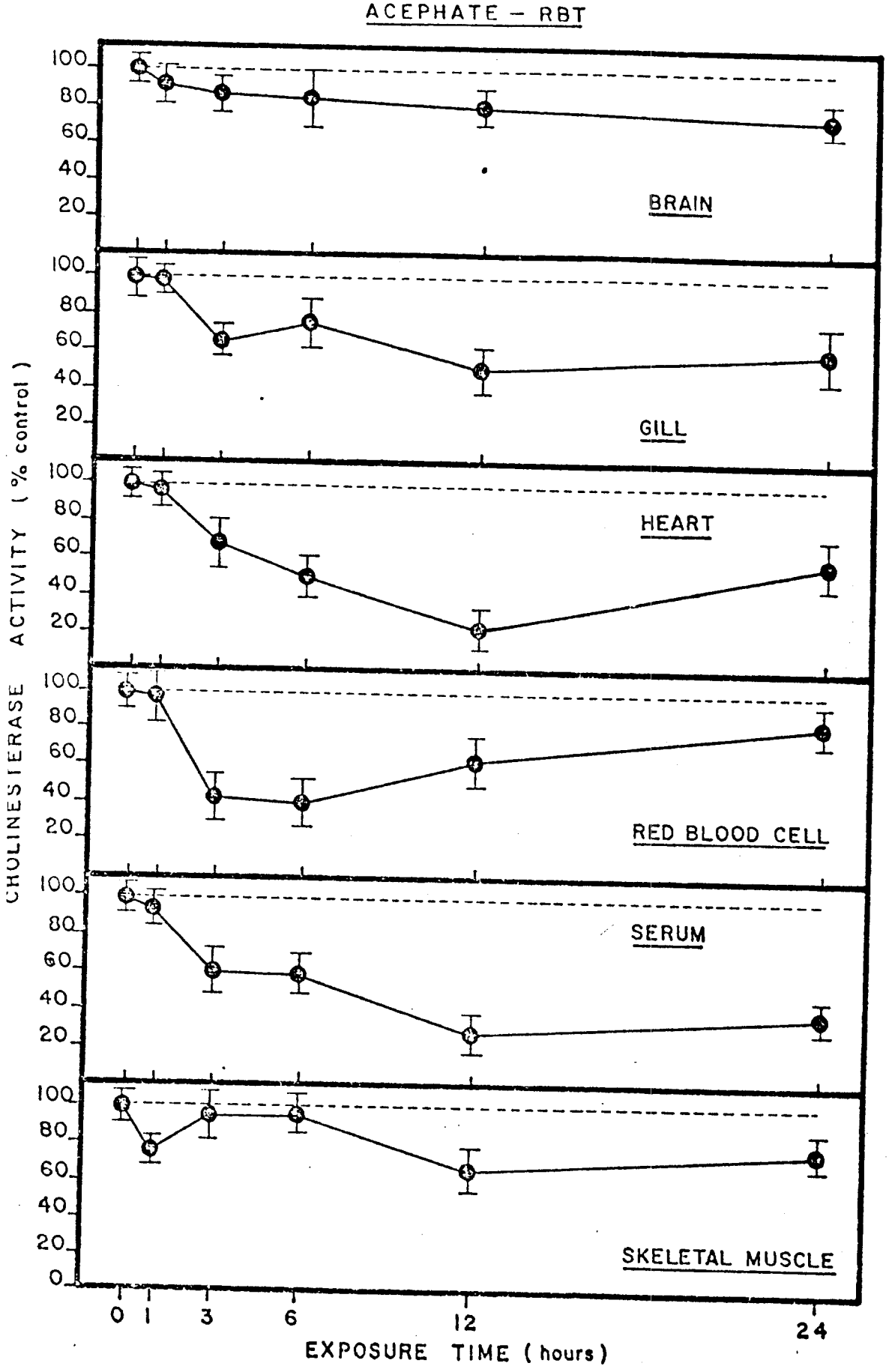
2.3.1. Acephate: The inhibition of cholinesterase (ChE) in brain, gill, heart, red blood cell, serum and skeletal muscle from rainbow trout exposed to acephate at 2000 mg/L is summarized in Table 11 and Figure 13. Brain enzyme activity decreased to 89% of control after 1 hour of exposure and continuously decreased to 85, 84, 79 and 72% of control at 3, 6, 12 and 24 hours, respectively. Gill enzyme activity of treated fish is not much affected at the first hour having an activity of 98% of control but decreased to 66% of control at 3 hours and recovered to 80% of control at 6 hours, but decreased again to 52 and 63% of control at 12 and 24 hours, respectively. The enzyme activity in the heart decreased continuously from 91 to 25% of control from 1 hour to 12 hours after exposure but increased to 74% of control at 24 hours. Red blood cell enzyme activity followed the same pattern as heart enzyme activity and dropped from 99% of control to 40% of control from 1 hour to 6 hours and increased to 64 and 90% of control at 12 and 24 hours respectively. Serum ChE activity decreased from 93% to 29% after 12 hours and increased to 41% of control at 24 hours. ChE activity in skeletal muscle varied between 65 to 96% of control.

In summary, acephate inhibited ChE activity in most tissues of rainbow trout especially in gill, heart, red blood cell and serum but most enzyme activity showed a recovery after 12 hours. Brain and skeletal muscle enzyme activities were not inhibited by acephate to the degree seen in other tissues. Figure 13 shows the enzyme activity of various tissues of rainbow trout at different times of exposure to acephate at 2000 mg/L.

Table 11. Cholinesterase (ChE) activity (μmoles ACh hydrolysed/mg protein/hr) of the mean of 3 fish ± S.E., in various tissues of rainbow trout exposed to acephate at 2000 mg/L.

Exposure Time (hr)		Brain	Gill	Heart	Red Blood Cell	Serum	Skeletal Muscle
0 hr	Mean±S.E.	13.22±0.20	0.32±0.08	1.66±0.08	2.80±0.19	2.35±0.15	5.06±0.11
	Range	12.55-13.87	0.24-0.43	1.40-1.94	2.33-3.45	2.00-2.76	4.72-5.46
	% control	100.00	100.00	100.00	100.00	100.00	100.00
+1 hr	Mean±S.E.	11.79±0.18	0.31±0.01	1.51±0.11	2.79±0.41	2.18±0.05	3.84±0.10
	Range	11.41-12.17	0.30-0.32	1.28-1.74	1.93-3.66	2.07-2.29	3.62-4.06
	% Control	89.20	98.10	91.18	99.60	92.88	75.92
+3 hr	Mean±S.E.	11.24±0.36	0.21±0.01	1.16±0.16	1.22±0.01	1.45±0.12	4.85±0.36
	Range	10.48-12.00	0.19-0.22	0.82-1.49	1.07-1.38	1.19-1.71	4.07-5.62
	% Control	85.04	65.93	70.05	43.66	61.68	95.85
+6 hr	Mean±S.E.	11.16±0.57	0.25±0.02	0.89±0.09	1.13±0.25	1.43±0.08	4.84±0.08
	Range	10.34-12.38	0.22-0.29	0.70-1.07	0.60-1.65	1.26-1.60	4.67-5.00
	% Control	84.43	80.17	54.04	40.27	60.93	95.66
+12 hr	Mean±S.E.	10.46±0.49	0.16±0.01	0.43±0.04	1.79±0.17	0.68±0.05	3.32±0.15
	Range	9.41-11.51	0.15-0.18	0.33-0.52	1.43-2.16	0.57-0.79	3.00-3.64
	% Control	79.15	51.69	25.87	63.97	28.99	65.64
+24 hr	Mean±S.E.	9.59±0.37	0.20±0.01	1.23±0.04	2.53±0.04	0.97±0.01	3.73±0.10
	Range	8.81-10.37	0.19-0.21	1.14-1.32	2.45-2.62	0.96-0.98	3.51-3.94
	% Control	72.56	63.29	74.37	90.42	41.11	73.74

Figure 13. Cholinesterase activity (percent of control) in brain, gill, heart, red blood cell, serum and skeletal muscle of adult rainbow trout following exposure to acephate at 2000 mg/L. Each point represents mean values of 3 fish with standard errors.


ACEPHATE – RBT
CHOLINESTERASE ACTIVITY (% control)
100
80
60
40
20
0
BRAIN
GILL
HEART
RED BLOOD CELL
SERUM
SKELETAL MUSCLE
0
1
3
6
12
24
EXPOSURE TIME (hours)

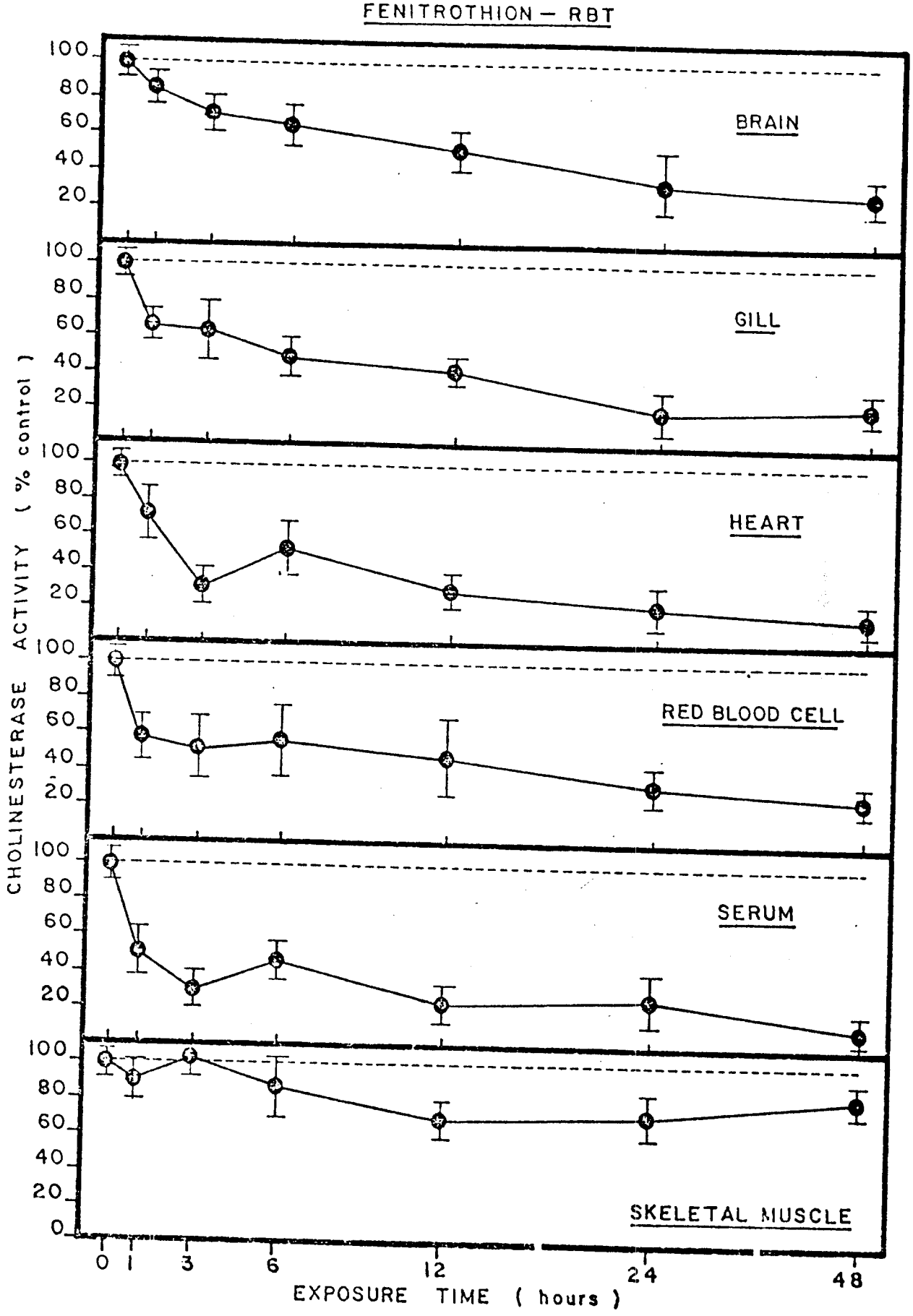
2.3.2. Fenitrothion: ChE inhibition in brain, gill, heart, red blood cell, serum and skeletal muscle of rainbow trout exposed to fenitrothion at 2.0 mg/L is presented in Table 12 and Figure 14. Brain ChE activity decreased after the first hour of exposure to 87% of control and continuously decreases to 77, 69, 54, 35 and 33% of control at 3, 6, 12, 24 and 48 hours respectively. Gill enzyme activity decreased to 65% of control after 1 hour and kept decreasing to 22% of control after 48 hours. Enzyme activity in the heart also decreased faster at the first 3 hours to 34% of control, recovered to 55% at 6 hours, but decreased again to 30, 23 and 18% of control at 12, 24 and 48 hours respectively. Red blood cell enzyme activity followed the same trend as enzyme in the heart with a relatively fast drop to 59% of control after 1 hour and a continuous decrease to 28% of control at 48 hours. Serum enzyme activity decreased to 50% and 32% of control after 1 and 3 hours of exposure and recovered at 6 hours to 47% of control but decreased afterward to 12% of control at 48 hours. ChE activity in skeletal muscle was not dramatically affected, having activity in the range of 71 to 105% of control.

In summary, fenitrothion caused a great decrease in enzyme activity in most tissues except skeletal muscle after 1 hour of exposure. The activity of enzyme in gill, heart, red blood cell and serum follow the same pattern. Figure 14 shows the enzyme activity in various tissues of rainbow trout after exposure to fenitrothion at 2.0 mg/L.

Table 12. Cholinesterase (ChE) activity (μmoles ACh hydrolysed/mg protein/hr) of the mean of 3 fish ± S.E., in various tissues of rainbow trout exposed to fenitrothion at 2.0 mg/L.

Exposure Time (hr)		Brain	Gill	Heart	RBC	Serum	Skeletal Muscle
0 hr	Mean±S.E.	15.39±0.03	0.24±0.01	1.27±0.04	2.93±0.05	2.45±0.10	5.77±0.09
	Range	15.30-15.51	0.22-0.27	1.17-1.43	2.79-3.17	2.05-2.79	5.40-6.07
	% Control	100.00	100.00	100.00	100.00	100.00	100.00
+1 hr	Mean±S.E.	13.49±0.30	0.16±0.01	0.92±0.16	1.72±0.24	1.21±0.12	5.21±0.34
	Range	12.93-14.17	0.14-0.19	0.62-1.28	1.14-2.05	0.96-1.47	4.49-5.94
	% Control	87.64	65.44	72.53	58.86	49.48	90.32
+3 hr	Mean±S.E.	11.81±0.78	0.15±0.02	0.43±0.09	1.52±0.36	0.78±0.09	6.06±0.22
	Range	10.53-13.68	0.10-0.21	0.21-0.55	0.93-2.38	0.65-1.00	5.70-6.60
	% Control	76.73	63.20	34.04	51.77	31.62	105.0
+6 hr	Mean±S.E.	10.63±0.50	0.12±0.02	0.69±0.20	1.71±0.45	1.16±0.04	5.04±0.68
	Range	9.83-11.84	0.08-0.15	0.41-1.18	0.60-2.31	1.09-1.24	3.76-6.62
	% Control	69.06	48.57	54.72	58.44	47.43	87.38
+12 hr	Mean±S.E.	8.44±0.26	0.10±0.01	0.38±0.05	1.38±0.47	0.58±0.07	4.12±0.66
	Range	7.84-8.95	0.09-0.12	0.29-0.50	0.26-2.19	0.41-0.71	2.59-5.35
	% Control	54.81	43.06	30.11	47.06	23.75	71.48
+24 hr	Mean±S.E.	5.46±1.34	0.05±0.02	0.30±0.09	1.04±0.18	0.63±0.25	4.31±0.74
	Range	2.51-8.20	0.01-0.10	0.08-0.42	0.65-1.41	0.10-1.17	2.49-5.35
	% Control	35.49	21.83	23.43	35.49	25.67	74.72
+48 hr	Mean±S.E.	5.03±0.03	0.06±0.01	0.23±0.04	0.84±0.10	0.30±0.06	4.87±0.19
	Range	4.98-5.09	0.05-0.07	0.13-0.32	0.62-1.05	0.17-0.43	4.50-5.31
	% Control	32.70	22.99	17.94	28.52	12.28	84.49

Figure 14. Cholinesterase activity (percent of control) in brain, gill, heart, red blood cell, serum and skeletal muscle of adult rainbow trout following exposure to fenitrothion at 2.0 mg/L. Each point represents mean values of 3 fish with standard errors.


FENITROTHION – RBT
BRAIN
GILL
HEART
RED BLOOD CELL
SERUM
SKELETAL MUSCLE
CHOLINESTERASE ACTIVITY (% control)
100
80
60
40
20
0
0
1
3
6
12
24
48
EXPOSURE TIME (hours)

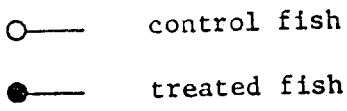
2.4 Determination of serum electrolyte

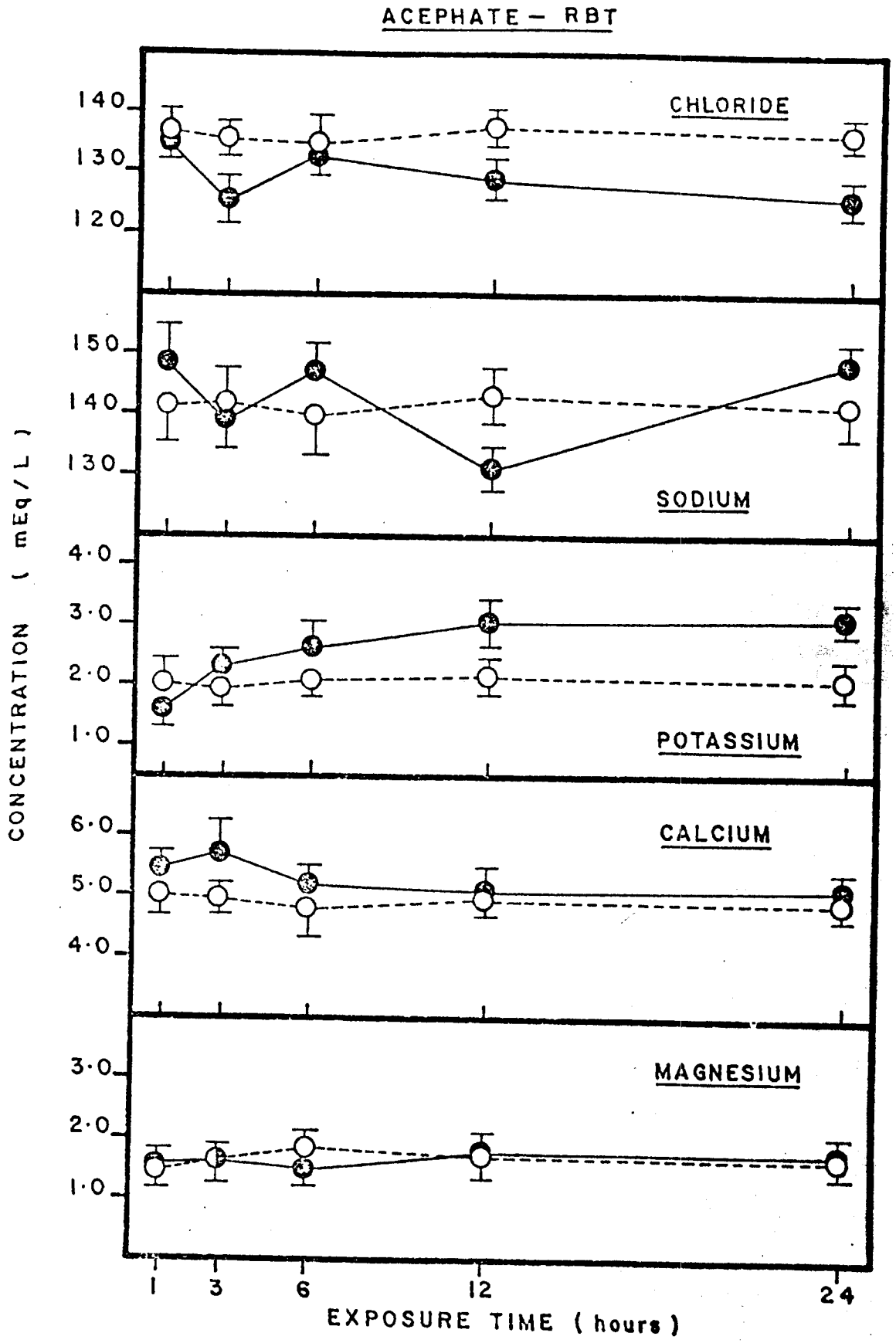
2.4.1. Acephate: Concentrations as mEq/L of serum chloride (Cl^-), sodium (Na^+), potassium (K^+), calcium (Ca^{++}) and magnesium (Mg^{++}) of control and treated fish exposed to acephate at 2000 mg/L at each exposure time are presented in Table 13. Serum Cl^- of the control fish varied between 135.1 ± 2.9 mEq/L to 138.6 ± 1.5 mEq/L during the exposure period. Serum Cl^- of treated fish decreased from 135.1 ± 1.3 mEq/L after 1 hour to 126.4 ± 2.1 mEq/L after 3 hours of exposure and recovered slightly to 132.7 ± 1.1 mEq/L after 6 hours but decreased significantly than the control ($p \leq 0.05$) to 126.4 ± 0.9 mEq/L after 24 hours of exposure. Serum Na^+ concentration of control fish varied between 138.0 ± 5.8 mEq/L to 144.8 ± 2.9 mEq/L during the exposure period. Serum Na^+ of treated fish varied inconsistently between 131.9 ± 1.9 mEq/L to 148.7 ± 4.0 mEq/L during exposure. The pattern with respect to Na^+ changes appeared to be one of the elevation and then depression but values were not significantly ($p \leq 0.05$) different from the controls. Serum K^+ of the treated fish increased from 1.6 ± 0.1 mEq/L after 1 hour to 2.3 ± 0.1, 2.6 ± 0.2, 3.1 ± 0.2 and 3.1 ± 0.1 mEq/L after 3, 6, 12 and 24 hours after exposure, compared with controls which varied between 1.9 ± 0.2 mEq/L to 2.2 ± 0.2 mEq/L during the exposure period. The trend over the experimental period was clearly toward an increase in serum level of this ion. Serum Ca^{++} concentration of treated fish varied between 5.2 ± 0.2 mEq/L to 5.8 ± 0.4 mEq/L and was not significantly ($p \leq 0.05$) different from control values which ranged between 4.4 ± 0.4 to 5.0 ± 0.2 mEq/L. Serum Mg^{++} concentration of treated fish appeared to be relatively stable with fluctuations about the control values and showed no marked tendency to increase or decrease. Figure 15 demonstrate

Table 13. Concentrations of serum electrolyte (mEq/L) of control and treated fish exposed to acephate at 2000 mg/L (values given are the mean of 3 fish ± S.E. and range)

Exposure Time	Cl^-		Na^+		K^+		Ca^{++}		Mg^{++}	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1 hr	137.0±2.7 (133.1-139.3)	135.1±1.3 (132.3-138.0)	140.1±4.2 136.9-146.1)	148.4±4.6 (140.9-149.5)	2.0±0.3 (1.8-2.3)	1.6±0.1 (1.4-1.7)	5.0±0.2 (4.8-5.1)	5.6±0.1 (5.5-5.7)	1.4±0.1 (1.3-1.7)	1.5±0.1 (1.5-1.6)
3 hr	136.2±1.5 (134.2-137.7)	126.4±2.1 (122.0-130.8)	141.9±3.9 (136.9-146.4)	137.7±3.7 (129.1-144.3)	1.9±0.2 (1.6-2.1)	2.3±0.1 (2.0-2.6)	4.8±0.1 (4.6-4.9)	5.8±0.4 (5.1-6.6)	1.7±0.2 (1.4-1.9)	1.7±0.1 (1.6-1.8)
6 hr	135.1±2.9 (133.3-136.0)	132.7±1.1 (130.3-135.1)	138.0±5.8 (136.9-144.8)	146.9±3.9 (138.7-155.2)	2.2±0.1 (2.1-2.4)	2.6±0.2 (2.4-2.9)	4.4±0.4 (3.2-4.2)	5.3±0.1 (5.0-5.6)	1.9±0.1 (1.8-2.0)	1.5±0.1 (1.3-1.7)
12 hr	138.6±1.5 (136.8-140.9)	129.8±2.5 (124.6-135.1)	144.8±2.9 (134.9-151.7)	131.9±1.9 (127.8-136.1)	2.2±0.1 (2.1-2.4)	3.1±0.2 (2.8-4.0)	4.9±0.1 (4.8-5.1)	5.2±0.2 (4.8-5.6)	1.7±0.2 (1.4-1.9)	1.8±0.1 (1.6-2.0)
24 hr	137.2±1.4 (135.2-138.7)	126.4±0.9 (124.5-128.3)	140.7±4.0 (136.4-146.1)	148.7±1.5 (139.6-150.0)	2.2±0.1 (1.9-2.4)	3.1±0.1 (3.1-3.2)	4.8±0.1 (4.8-4.9)	5.3±0.1 (5.2-5.4)	1.6±0.1 (1.4-1.8)	1.7±0.1 (1.6-1.9)

Figure 15. Changes of serum chloride, sodium, potassium, calcium and magnesium concentrations (mEq/L) in adult rainbow trout following exposure to acephate at 2000 mg/L. Each point represents mean values of 3 fish with standard errors.




ACEPHATE – RBT
CHLORIDE
SODIUM
POTASSIUM
CALCIUM
MAGNESIUM
140
130
120
150
140
130
4·0
3·0
2·0
1·0
6·0
5·0
4·0
3·0
2·0
1·0
CONCENTRATION (mEq/L)
1
3
6
12
24
EXPOSURE TIME (hours)

the changes in serum electrolytes of treated and control fish after exposure to acephate at each exposure time.

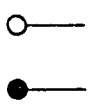
2.4.2. Fenitrothion: The concentrations of serum Cl^-, Na^+, K^+, Ca^{++} and Mg^{++} of control and treated fish exposed to fenitrothion are summarized in Table 14. Serum Cl^- of the treated fish fluctuated between 134.3 ± 0.1 mEq/L to 141.0 ± 1.6 mEq/L during the first 12 hour period and then decreased to 126.4 ± 2.6 mEq/L and 126.0 ± 2.4 mEq/L after 24 and 48 hours of exposure, but were not significantly different ($p \leq 0.05$) from the controls which varied between 135.1 ± 9.3 mEq/L to 139.2 ± 4.3 mEq/L during the exposure period. Na^+, like Cl^-, fluctuated about the control values from 145.3 ± 3.9 mEq/L to 15.0 ± 1.5 mEq/L for the first 12 hours and decreased slightly, to 136.7 ± 4.2 mEq/L and 139.7 ± 4.6 mEq/L after 24 and 48 hours. By contrast, serum K^+ of treated fish increased from 3.1 ± 0.2 mEq/L after 1 hour of exposure to 3.2 ± 0.3, 3.2 ± 0.3 and 3.4 ± 0.3 mEq/L after 3, 6 and 12 hours respectively and continued to increase until significantly different ($p \leq 0.05$) than the controls to 4.1 ± 0.2 and 4.7 ± 0.2 mEq/L after 24 and 48 hours. Serum Ca^{++} of treated fish did not change significantly ($p \leq 0.05$) from the controls and varied between 4.3 ± 0.2 mEq/L to 5.3 ± 0.1 mEq/L. Serum Mg^{++} showed the same pattern as observed in the acephate experiment with fluctuations about the control values until the end of the experiment. Figure 16 shows the changes in serum electrolytes of control and treated fish after exposure to fenitrothion at each exposure time.

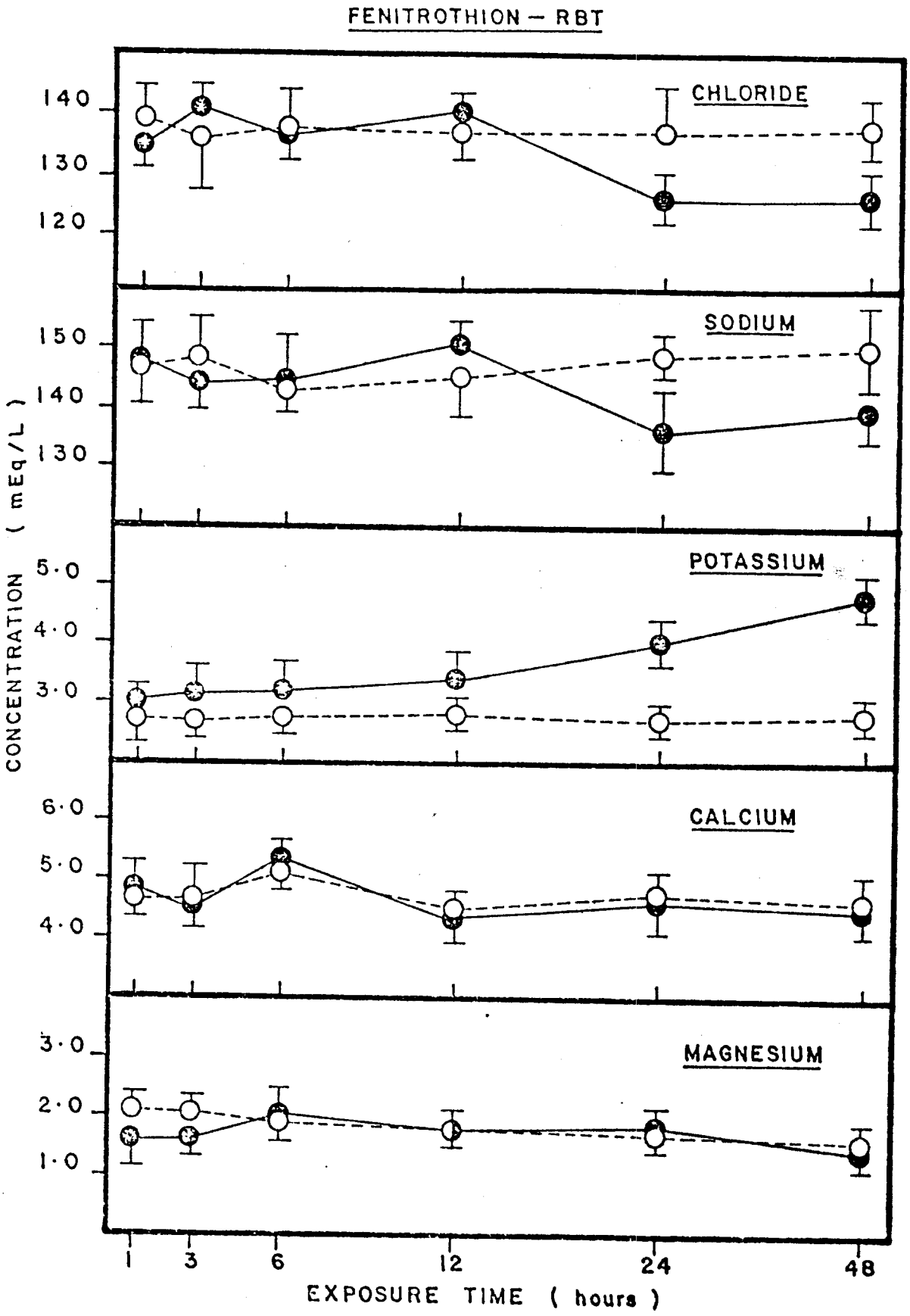
In summary, both acephate and fenitrothion produced an increase in serum K^+ concentration and a slight decrease in serum Cl^- concentration of rainbow trout.

Table 14. Concentrations of serum electrolytes (mEq/L) of control and treated fish exposed to fenitrothion at 2.0 mg/L (values given are the mean of 3 fish ± S.E. and range).

Exposure Time	Cl^-		Na^+		K^+		Ca^{++}		Mg^{++}	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1 hr	139.2±4.3 (135.1-145.7)	134.3±0.7 (132.9-136.0)	146.9±4.4 (140.8-151.2)	148.4±4.6 (137.4-155.6)	2.7±0.1 (2.6-2.8)	3.1±0.2 (2.7-3.5)	4.7±0.2 (4.4-4.9)	4.9±0.3 (4.4-5.7)	2.2±0.1 (2.1-2.4)	1.6±0.2 (1.2-1.9)
3 hr	135.1±9.3 (123.4-146.2)	141.0±1.6 (138.9-144.9)	148.0±4.8 (142.6-154.5)	145.3±3.9 (137.4-153.9)	2.7±0.1 (2.5-2.8)	3.2±0.3 (2.7-3.8)	4.7±0.4 (4.2-5.2)	4.6±0.3 (4.0-5.3)	2.2±0.1 (2.0-2.3)	1.6±0.1 (1.6-1.7)
6 hr	138.2±4.1 (132.5-142.0)	136.9±2.7 (124.0-146.9)	143.4±2.2 (141.1-146.3)	145.5±5.4 (133.0-155.6)	2.8±0.1 (2.6-2.9)	3.2±0.3 (2.8-3.8)	5.1±0.1 (4.9-5.2)	5.3±0.1 (5.0-5.5)	1.9±0.1 (1.6-2.3)	2.1±0.2 (1.7-2.5)
12 hr	136.4±3.1 (133.3-140.6)	140.1±0.9 (138.9-142.3)	146.5±4.4 (141.3-152.6)	150.0±1.5 (143.9-153.5)	2.8±0.1 (2.7-2.9)	3.4±0.3 (3.0-4.0)	4.5±0.1 (4.2-4.9)	4.3±0.2 (3.9-4.7)	1.8±0.2 (1.6-2.0)	1.8±0.2 (1.5-2.2)
24 hr	136.1±5.3 (128.6-140.3)	126.4±2.6 (110.0-137.1)	148.3±2.0 (145.6-150.4)	136.7±4.2 (131.3-146.9)	2.8±0.1 (2.6-2.9)	4.1±0.2 (3.8-4.5)	4.8±0.2 (4.6-5.0)	4.7±0.3 (4.2-5.3)	1.6±0.1 (1.5-1.8)	1.8±0.1 (1.6-2.0)
48 hr	137.1±3.5 (134.0-142.0)	126.0±2.4 (114.9-137.1)	150.6±6.4 (147.2-156.9)	139.7±4.6 (130.0-149.6)	2.8±0.1 (2.6-2.9)	4.7±0.2 (4.6-4.8)	4.7±0.3 (4.3-5.1)	4.6±0.3 (4.0-5.3)	1.6±0.1 (1.5-1.8)	1.5±0.1 (1.3-1.6)

Figure 16. Changes of serum chloride, sodium, potassium, calcium and magnesium concentration (mEq/L) in adult rainbow trout following exposure to fenitrothion at 2.0 mg/L. Each point represents mean values of 3 fish with standard error.




FENITROTHION – RBT
CHLORIDE
SODIUM
POTASSIUM
CALCIUM
MAGNESIUM
140
130
120
150
140
130
5·0
4·0
3·0
6·0
5·0
4·0
3·0
2·0
1·0
CONCENTRATION (mEq/L)
1
3
6
12
24
48
EXPOSURE TIME (hours)

DISCUSSION

1. Effects of temperature on acute lethality and ChE inhibition

1.1. Acute lethality studies

The results from these studies indicate that temperature stress alters the toxicity of each OP insecticide, especially that of fenitrothion, to rainbow trout. The effects of temperature stress on the susceptibility of fish, as indicated by LC_{50} and MST values, (Table 2 and 3) were observed in the first 24 hour period but were less pronounced after 48 hours and no significant difference was observed after 96 hours of exposure. The difference in toxicity after 24 hour exposure may be caused by temperature induced stresses. In these experiments, fish were subjected to abrupt temperature changes from the acclimation temperature of 15°C to test temperatures at 8° and 22°C, referred to as cold and heat stresses, for 48 hours before starting the experiment.

The same pattern of temperature effects observed in this study was also reported by several investigators in other toxicants in various fish species. Toxicity of zinc to rainbow trout is greater at 21.5°C than at 13.5°C in short time intervals but the toxicity is not different after 3 days (Lloyd, 1960). Thatcher et al. (1976) studying the effects of chlorine and temperature on juvenile brook trout (Salvelinus fontinalis) observed the difference in mortality was exhibited prior to 48 hours of exposure but there was no significant difference between the 96-hour mortality values between experiments run at 10° and 15°C. They also observed that the greater the temperature shock, the higher the mortality during the early exposure periods.

The rate of insecticide uptake by fish can be influenced by temperature through its effect on the respiratory system. The branchial

respiratory surfaces of fish are probably the most important route of direct insecticide uptake because they are permeable and have a large surface area. Toxicant uptake through the gills can increase with an increase in respiration rate which can be induced by an increase in the temperature of the aquatic medium (Warren, 1971). Rainbow trout increased both frequency and amplitude of ventilation with a rise in temperature (Hughes and Roberts, 1970; Heath, 1973; Heath and Hughes, 1973) and thus increased ventilation volume (Randall and Cameron, 1973). Many investigators also reported the effects of temperature on the accumulation of toxic substances from water by fish in both short and long term periods. The accumulation of p',p'DDT by mosquitofish (Gambusia affinis) (Murphy and Murphy, 1971) and mercuric chloride by rainbow trout (Macleod and Pessah, 1973) increased with an increase in temperature. Reinert et al. (1974) who studied the effects of temperature on the accumulation of mercuric chloride and p',p'DDT by rainbow trout over a long period also found the same result and suggested that the extent of an increase in accumulation would depend on factors such as the amount of increase in water temperature, the amount of time fish are in the the warmer water, the initial concentration of toxic materials in the fish and the rate of excretion.

From these observations therefore, slowing metabolism by lowering the temperature may have allowed a longer survival time by reducing ventilation rate and consequently insecticide uptake. Similarly, metabolic acceleration following an increase in temperature may have shortened survival time by accelerating insecticide uptake.

The physicochemical properties of substances that influence their movement across biological membranes are fairly well established and

and include lipid solubility, water solubility, degree of ionization, chemical stability and molecular weight (LeFebre, 1972). Many compounds pass across membranes by passive diffusion at a rate determined by the lipid solubility of the compound and proportional to its concentration gradient across the membrane (Schanker, 1962; Korolkovas, 1970). Compounds that are lipid-insoluble and of very small molecular size can pass through membranes by simple diffusion through minute water-filled pores and the rate of passage is proportional to concentration difference across membrane (Kruhoffer, 1961).

Fenitrothion is soluble in most organic solvents but of low solubility in water (Sumitomo Chemical Company, 1963; Muramoto, 1976). Zitko and Cunningham (1974) reported the solubility of fenitrothion in water is of the order of 20 mg/L. On the contrary, acephate which has very high solubility in water (about 65 percent) and relatively low solubility in organic solvents (less than 5 percent) (Chevron Chemical Company, 1973) is a lipid-insoluble substance.

The effects of temperature stress on the respiration rate of fish, therefore, will have greater influence on the uptake of fenitrothion which is a lipid-soluble compound than acephate which is a water soluble compound. Acephate, having pKa of 8.3, was ionized about 97 percent at the pH level of the tested water (Table 2) would probably penetrate through membranes at a much slower rate and would require higher concentrations than fenitrothion to produce a significant concentration gradient across the membranes. This fact was observed on the survival times of fish exposed to fenitrothion which are more affected by temperature stresses than acephate (Table 3). In general, fish died faster in heat stress and slower in cold stress.

Heat stress shortens the survival times of fish exposed to several toxicants has also been reported by many investigators. A temperature increase of 1.5°C per 10 minutes reduced the survival time of bluegills (Lepomis macrochirus) exposed to lethal and sublethal concentrations of zinc (Burton et al. 1972). Hodson and Sprague (1975) who studied the effect of heat and cold stresses on the acute toxicity of zinc to atlantic salmon (Salmo salar) reported that moderate (8°C) and severe (16°C) heat stresses shortened time to mortality and the opposite results were observed in cold stresses. They also observed that heat and cold stresses changed the slopes of the mortality curves.

The slopes of mortality curves in acephate experiments are decreased slightly as temperature decreases (Figure 3) but are not significantly different ($p \leq 0.05$). In fenitrothion experiments, the slopes of mortality curves are decreased significantly ($p \leq 0.05$) when temperature decreases (Figure 4).

Nonparallelism of the mortality curves observed in fenitrothion experiments at different temperatures suggests that different mechanisms of toxic action are involved. The activation of fenitrothion by MFO enzyme system to fenitrooxon is probably the major factor that causes the changes in the slopes of mortality curves since fenitrooxon is more toxic to fish than fenitrothion and therefore the mortality rate is changed. The slopes of mortality curves in acephate studies are not changed indicating that the mode of toxic action of acephate was not affected by temperature.

The phosphorothioate insecticides (eg. fenitrothion) having the basic structure of P=S are not potent ChE inhibitors and usually undergo

biotransformation processes by MFO enzyme system in the liver to the more potent ChE inhibitors or their oxygen analogs having the basic structure of P=O eg. fenitrooxon. Klaverkamp et al. (1976) indicated that rainbow trout can convert fenitrothion to a more potent ChE inhibitor, probably fenitrooxon. Therefore, increase in temperature may increase the rate of biotransformation of fenitrothion to fenitrooxon which is more toxic (Eto, 1974) resulting in the increase fish mortality. Acephate, on the contrary, does not have to undergo the biotransformation process by the liver enzyme system and therefore was not much affected by temperature.

The Q_{10} values of the rate of mortality of acephate ranged between 0.68 to 2.02 (Table 4) with a mean value of about 1.3 (ie. a 10°C increase in temperature caused a 1.3 fold increase in the rate of mortality). Shifrer et al. (1974) suggested that Q_{10} values for physical processes generally range between 1.1 to 1.2, and the Q_{10} values from acephate experiments are close to this range. The physical processes ie. diffusion and/or osmotic pressure were probably involved in the effect of temperature on toxicity of acephate. The rate that a substance (molecule) moves through a membrane by diffusion is believed to increase by approximately 10 percent with 10°C rise in temperature (Middlebrooks et al. 1973).

The Q_{10} values for the rate of mortality from difference concentrations of fenitrothion ranged from 1.11 to 5.57 (Table 4) with a mean value of about 3.3 (ie. a 10°C increase in temperature caused a 3.3 fold increase in the rate of mortality). Shifrer et al. (1974) suggested that when a chemical reaction is involved in the toxic action, Q_{10} values might range from 2 to 4 or higher. Therefore, for fish exposed to fenitrothion it is possible that fenitrothion toxicity is influenced

by temperatures in a similar way that enzyme kinetics are controlled as also is suggested from the changes of slope of the mortality curve.

The difference in the acute lethality between acephate and fenitrothion to rainbow trout fingerlings of about 600 to 1000 times observed from this study is quite interesting since the in vitro studies of brain ChE inhibition in rainbow trout by Klaverkamp et al. (1975) indicated that the difference in potency of acephate and fenitrothion for inhibiting brain ChE is relatively small. The pI 50 values (negative log of the concentration producing 50 percent brain ChE inhibition) for acephate and fenitrothion were observed at 0.9 and 1.1 respectively. The difference in the in vivo situation could be attributed to the variation in the movement of acephate and fenitrothion across biological membranes in fish to reach and react with the target receptor and/or the capacity of fish liver enzyme system to activate fenitrothion to fenitrooxon which is very potent ChE inhibitor. The pI 50 value of fenitrooxon was observed at 5.8 (Klaverkamp et al. 1975) which is about 4.3×10^4 and 0.8×10^5 times greater in potency than fenitrothion and acephate respectively.

1.2 ChE inhibition in brain and skeletal muscle

The results observed from these studies indicate that the inhibition of ChE in brain and skeletal muscle in rainbow trout fingerlings by acephate and fenitrothion are not correlated with mortality. Weiss (1958, 1959, 1961) suggested that death will occur when brain ChE activity drops to 40-70 percent of normal activity. Coppage (1972) and Coppage and Mathews (1974) suggested that 80 percent inhibition of brain ChE activity should be the critical level in fish in short term OP insecticide

poisoning. The results from this experiment, however, do not support these suggestions since the brain enzyme activity in surviving fish were, in some cases, lower than that level while in dead fish brain enzyme activity was sometime higher than that level. Gibson et al. (1969) observed the same results in bluegill (Lepomis macrochirus) with parathion and concluded that mortality and recovery from OP insecticide poisoning are not necessarily related to the degree of brain ChE inhibition.

Coppage (1971) proposed a method for in vivo ChE inhibition study using a pH stat method which was applied in tests comparing in vivo brain ChE inhibition and toxicity of several OP insecticides in Sheepshead minnows (Cyprinodon variegatus) and found ChE inhibition was correlated with exposure time and observed toxicity. He concluded that the confusing relationship between mortality and degree of in vivo ChE inhibition reported by Gibson et al. (1969) could be in the methodology, since Gibson et al. (1969) used the spectrophotometric method which is subject to several limitations and possible sources of error.

While agreement concerning the so-called critical levels of ChE activity that will be lethal to fish is lacking, there is a possible explanation that answers the question of high level of ChE activity in dead fish, especially those exposed to high concentrations of insecticide, in this study. Recent concepts of cholinergic pharmacology have recognized that tissue ChE can be divided into 2 pools, extracellular and intracellular pools, and that only the former enzyme pool seems related to pharmacologic function whereas the intracellular pool is the nonfunctional or reserve enzyme (Koelle, 1970b; Silver, 1974). Previous studies on

nerves, brain, striated muscle and iris demonstrated that external ChE or functional pool of enzyme which regulates responses to cholinergic drugs constitutes approximately 20 percent and internal ChE or non-functional enzyme constitutes approximately 80 percent of total enzyme activity, and that the pharmacologic effect of anti-ChE agents is apparently achieved by the inhibition of external or functional enzyme (McIsaac and Koelle, 1959; Hobbiger and Vojvodic, 1967; Harris et al. 1972). Diisopropylfluorophosphate (DFP), an OP insecticide, was found to have no pharmacologic effect on the diaphragm muscle of mammals if the external enzyme pool is protected from inhibition even when internal ChE is almost completely inhibited (Mittag et al. 1971). Consequently, when tissue homogenates are used to study drug effects on ChE, at least 80 percent of the enzyme assayed has no direct connection with cholinergic function (Ehrenpreis et al. 1970; Mittag et al. 1971). Harris et al. (1972) also concluded that while tissue homogenates can give more precise kinetic data, much of the enzyme measured in this way is nonfunctional ChE and it has no relation to drug response. They suggested a new radiometric technique and used it to study the effect of DFP on ChE activity and contractility of intact cat iris. They also reported that it is the inhibition of the surface or extracellular ChE of the iris, constituting approximately 18 percent of total tissue ChE, which is involved in potentiation of contractility induced by agonists. Therefore, it seems that the homogenization of brain and skeletal muscle samples used in the ChE analysis in this study may allow the nonfunctional ChE or intracellular pool to become available to the substrate used and may result in high enzyme activity even when the functional enzyme pool may be completely inhibited.

High ChE activity in dead fish was observed at high concentrations of insecticide, especially at 22°C test temperature in the fenitrothion study. This may be due to the less time that those fish stay in the test solutions, therefore the total ChE activity is still high but the functional enzyme pool may be inhibited. For the fish that died at lower concentrations and stay longer in the test solutions there is a lower total enzyme activity because the insecticides have more time to penetrate and pass through the cell membranes and then inhibit both functional and nonfunctional pools of enzyme.

The results observed from this study also demonstrated that surviving fish may have lower enzyme activity, in some cases, than the fish that died which suggested that adaptive or tolerance mechanisms may have developed in those fish. Mechanisms responsible for tolerance probably exist in fish that are capable of increasing the synthesis or reactivation of ChE and/or lowering the accumulated acetylcholine (ACh) levels in spite of a persisting or increasing depression of ChE activity. The recovery of skeletal muscle ChE in rat after 1 hour of exposure to melvinphos, an OP insecticide, is believed to be caused by the syntheses of new enzyme (Sharma et al. 1973).

The mechanism of tolerance by lowering ACh levels has been suggested and discussed by several investigators. Brain ACh levels of mammals were found to return toward normal level within a short time after anti-ChE treatment (Bignami et al. 1975). An explanation of this mechanism is that the high levels of the neurotransmitter (ACh) in the tissue inhibit the synthesis of the neurotransmitter itself as proposed by Kaita and Goldberg (1969) but the evidence is far from clear. Another possibility is that the long-term accumulation of high concentration of ACh

probably leads to a reduction of receptor sensitivity which in turn allows a recovery of function as reported by Overstreet et al. (1972).

Therefore, it seems that surviving fish may be capable of using any or all of these mechanisms to tolerate the accumulation of ACh and lowering of ChE activity in the brain tissue. It is also known that after OP insecticide treatment brain ACh levels of animals tested can return toward normal level much faster than the ChE activity and it appears that the return of brain ACh levels correlated fairly well with the disappearance of intoxication symptoms, but shows little relationship with ChE activity (Bignami, et al. 1975). However, no attempt was made in this study to measure the ACh levels in the brain of fish and the data presently available do not allow a quantitative evaluation of the relative role of the above mechanisms.

2. Cardiovascular/respiratory responses and ChE inhibition

2.1. Cardiovascular and respiratory responses

The decrease in heart rates in fish exposed to acephate and fenitrothion is characteristic of ChE inhibitor poisoning as observed by many investigators (Matton and Lattam, 1969; Majewski and Klaverkamp, 1975; Klaverkamp et al. 1976; Lunn et al. 1976). Acephate and fenitrothion, by inhibiting cardiac or vagal ChE, may cause an accumulation of ACh and produce bradycardia in fish. Bradycardia has been suggested to function as a mechanism that could alter the dynamics of blood flow at the vicinity of the respiration surface. Holeton and Randall (1967) indicated that the slower heart rate may facilitate oxygen uptake by permitting the blood to remain in the gill lamellae for a longer period of time.

The occasional "locking" of heart beat in fish to a specific phase of the respiratory cycle, a phenomenon known as synchrony (Shelton and Randall, 1962) was observed in some fish in this study. This relationship has been reported by several investigators as a response to stresses, such as anesthetization (Shelton and Randall, 1962), temperature change (Heath and Hughes, 1971), and recovery from surgery (Sutterlin, 1969). Randall and Smith (1967) reported that both the bradycardia and the cardiorespiratory synchrony are related to hypoxic conditions at the respiratory surface and both are maintained by inhibitory activity in the efferent cholinergic fibers contained within the cardiac ramus of the vagus. It has been proposed that synchrony functions to assure that sufficient oxygen is available to saturate the blood when it passes through the gills (Hughes, 1964; Randall and Smith, 1967). The relationship might provide synchronization of high flow rates in two pulsatile systems, blood and water, with the outcome being a moment to moment balance of ventilation and perfusion resulting in an increased efficiency in gas exchange. However, this relationship is inexact and of short duration as observed in this study and was confirmed not to be a dominent effect in fish (Weintraub and Mackay, 1975).

The increase in respiration rates and amplitudes and the decrease in heart rates in fish exposed to acephate and fenitrothion are similar to responses of fish exposed to hypoxia (Randall and Shelton, 1963; Randall and Smith, 1967; Hughes, 1973). These responses indicate that oxygen uptake may be affected by the insecticides and/or a lack of oxygen supply is delivered to the cells or tissues. The increased ventilation rates in treated fish and amplitudes in this experiment was not due to a reduction in oxygen concentration of the inspired water since oxygen

was supplied at near saturation level in each chamber by the use of an airstone as described previously. Hughes (1973) suggested that hypoxic condition in fish may be caused by several factors such as: malformation or paralysis of the normal ventilatory apparatus; interference with the diffusion exchange across the gill surfaces; the excessive shunting of venous blood or by-passing the respiratory exchange surfaces.

It seems likely in this experiment that the excessive shunting of venous blood or by-passing the respiratory exchange surfaces is the major process involved. Two filamental shunt models are based on observations in eels (Anguilla vulgaris) by Steen and Kruysse (1964) and in rainbow trout (Salmo gairdneri) by Richards and Fromm (1969) where flow from afferent to efferent filamental vessels was observed to be via (a) the flat lacuna secondary lamellae (respiratory pathway) and (b) a central sinus in the filamental body (non-respiratory filamental shunt). It has been assumed that regulation of vascular resistance is similar in fish and higher vertebrates (Satchell, 1971) and that the effects of cholinergic and adrenergic drugs and hormones on branchial resistance are qualitatively similar. Using different methods, Steen and Kruysse (1964) and Richards and Fromm (1969) observed that ACh increased filamental sinus blood flow (non-respiratory shunt) and epinephrine increased secondary lamellar blood flow which is the respiratory flow. Fromm et al. (1971) examined the effects of several chlorinated hydrocarbon and OP insecticides on the rate of fluid flow through isolated perfused rainbow trout gills and reported a decrease in flow rates at constant perfusion pressure which means that resistance to fluid flow through gills was increased. Bergman et al. (1974) also confirmed that the functional surface area of rainbow trout gills can be regulated by

changing perfusion pathway with adjustments in the relative vascular resistance across the different pathways. They also reported that ACh decreased functional gill surface area by causing the constriction of the afferent and efferent lamellar vessels reducing flow to lamellae and increasing overall branchial vascular resistance. Wood (1975) reported the presence of cholinergic receptors in the gills of rainbow trout which are muscarinic in nature and mediate vasoconstriction by innervation probably through vagal origin. Therefore, acephate and fenitrothion may produce hypoxic conditions in fish through the cholinergic receptors by increasing the branchial vascular resistance and reducing the functional gill surface area which in turn affected the respiratory gas exchange between the gill and water.

The ECG waveforms observed during these experiments demonstrated changes that are nearly identical to those observed in rainbow trout responses to stress of hypoxia as reported by Bahr (1968). The characteristics of changes in ECG waveforms in fish during exposure to acephate and fenitrothion in these experiments are also similar to the changes observed in mammalian heart under myocardial ischemic condition and myocardial electrolyte imbalance as described by Nasser (1970) and Goldman (1973). The characteristic change in ECG waveform during electrolyte imbalance eg. hyperkalemia (an increase in extracellular potassium) is described as an increase in magnitude of the T-wave by the appearance of a tall, slender, "tented" T-wave which at high levels of potassium will produce prolongation of the PR interval and the QRS duration. ECG changes during myocardial ischemia are transitory ST segment deviation and T-wave changes downward or T-wave inversion (Nasser, 1970; Goldman, 1973).

The characteristic changes in ECG waveforms described above were also observed in this experiment, therefore, it is probable that acephate and fenitrothion may produce myocardial ischemic conditions and electrolyte imbalance ie. hyperkalemia in the fish heart.

Myocardial ischemic condition can also cause a build-up of metabolites presumably due to an increase in anaerobic metabolism. Metabolites which can occur include lactic acid, carbon dioxide and perhaps others (Nasser, 1970). The hypoxic heart's lactic acid production from either glucose or endogenous glycogen is a well-documented fact in myocardial ischemia, and it has been widely recognized in mammals including man during cardiogenic shock (Shea et al. 1962). Holeton and Randall (1967) found that lactic acid will accumulate extracellularly in trout exposed to hypoxic conditions. However, no attempt was made in this study to measure the amount of lactic acid production in fish but it very likely occurred in these experiments. Experimental myocardial ischemia is also consistently characterized by potassium loss from the ischemic myocardium (Case et al. 1969; Gerlings et al. 1969). There is a linear relationship between myocardial lactate production and potassium loss, suggesting that the amount of potassium lost is directly related to the degree of ischemia (Case et al. 1969; Gerlings et al. 1969).

Potassium and calcium are the only major electrolytes considered to have independent effects upon the ECG response (Nasser, 1970). Changes in the sodium ion concentration that produce ECG changes are largely due to the modification of the effect of potassium ion concentration (Nasser, 1970). Hyperkalemia, an elevated extracellular potassium level, is the most lethal of electrolyte disorders in the heart (Nasser, 1970; Goldman, 1973). It has been indicated by Adrian (1956) that ex-

cessive amounts of extracellular potassium will cause hyperpolarization of neural membranes and eventually render the nerves functionless. It is possible that potassium will also accumulate extracellularly in fish under this experimental condition as will be discussed later in the serum electrolyte determination section.

It is interesting to note that fenitrothion produced an increase in cough frequency but acephate did not. Changes in cough frequency in fish have been used to indicate the presence of some toxicants in water (Schaumberg et al. 1967; Skidmore, 1970; Sparks et al. 1972). The increase in frequency of coughing is believed to be caused by mechanical irritation to the gill epithelium and, presumably, the cough reflex serves as a means of ridding the gills of foreign matter and eliminating clogging of the respiratory epithelium (Hughes and Shelton, 1958). Heavy metals are known to coagulate mucus secreted on the gills, and the cause of death was suggested as being due to hypoxia resulting from the lifting of the surface epithelium away from the vascular bed of the secondary lamellae with subsequent lengthening of the oxygen diffusion pathway (Skidmore, 1970). No evidence was observed in this study to indicate that fenitrothion or acephate caused mucus secretion in fish gills.

Few observations have been made on the neurological basis of coughing. Shelton (1959) showed that there are neurones situated in the anterior part of the medulla beneath the cerebellum of the tench (Tinca tinca) which were particularly responsible for co-ordination of the coughs and transection experiments tended to confirm this. Analysis of the relationship between coughing and ventilatory cycles also suggests independence with some coupling of the rhythms (Hughes and Morgan, 1973).

Therefore, if the coughing response is controlled by the nervous system as suggested, the possible explanation of the difference in coughing response to acephate and fenitrothion lies in their different physico-chemical properties. Fenitrothion, as a lipid-soluble compound, can penetrate through the blood-brain barrier and inhibit nerve function in the region of the hind brain. Acephate, on the other hand, is a lipid-insoluble compound and would not be effective in passing through the blood-brain barrier and reaching the site that controls the coughing response. The difference between fenitrothion and acephate in the effectiveness of their inhibition of brain ChE has been demonstrated and will be discussed in the next section.

An alternative explanation to the increase in cough response to fenitrothion may be gill irritation produced by fenitrothion. Fenitrothion may cause changes in the cell membrane at the gill epithelium and produce disruption of the cell membrane. However, no attempt was made in this study to examine histological changes in the gill tissues. Histological changes in the gills of rainbow trout larvae exposed to Dylox, an OP insecticide, were studied by Matton and Lattam (1969) who reported the gill arrangement was disrupted, the cell rows were no longer parallel, the elongated epithelial cells were distended with swollen nuclei and distorted blood cells were present in the mid-zone. This interpretation, however, still does not explain why acephate did not irritate the gills.

Davis (1973) suggested that coughing might interfere with proper oxygen uptake by reversing the flow of water over the gills, thereby interfering with the normal countercurrent exchange, which maintains a maximum oxygen gradient between water and blood. In addition, coughing may cause the gill filament tips, which interdigitate to be pulled apart,

so that water is not efficiently supplied to the second lamellae (Davis, 1973). The increase in the respiration rate and amplitude observed in fenitrothion experiments may also be a compensation for loss of respiratory efficiency during coughs.

From these observations, it is concluded that the cardiovascular/respiratory systems of fish are important sites of action for acephate and fenitrothion toxicity. Their toxic effects produced in fish could be caused by myocardial hypoxia induced by an increase in branchial vascular resistance and reduce gill functional surface areas which in-turn upset the oxygen uptake. It is suggested here that oxygenated-blood circulation (ie. coronary system) was severely reduced and perhaps even lacking in some tissues and thus the myocardial ischemic condition developed. However, no attempt was made in this study to measure other blood parameters such as blood pO_2, pCO_2, pH and lactate levels in treated fish during exposure to acephate and fenitrothion. Therefore, this proposed toxic action of acephate and fenitrothion in rainbow trout by producing internal hypoxia needs further investigation.

2.2 ChE inhibition in fish tissues

The results obtained from ChE studies indicate that acephate and fenitrothion caused a differential pattern of ChE inhibition in various tissues (Figures 13 and 14). The extent to which ChE was inhibited in different tissues depends upon the rate at which the chemical reaches the ChE and that depends on the physicochemical properties of both OP insecticides. The inhibition of ChE by acephate in most tissue studies except skeletal muscle is not significant after 1 hour of exposure but fenitrothion inhibited ChE significantly in most tissues except brain and

skeletal muscle after 1 hour. This result may be explained by the different rates of absorption and penetration of both compounds through the cell membrane to the target tissues.

Acephate, a water soluble and about 76% ionized (pKa 8.3) in normal blood pH of 7.8 (Bass and Heath, 1977), probably does not easily penetrate into the brain as suggested by the low ChE inhibition (about 28 percent inhibition in brain tissue after 24 hours of exposure at a concentration that is lethal to fish). Fenitrothion, a lipid soluble OP insecticide, however, can inhibit brain enzyme about 65 percent after 24 hours of exposure at a concentration that is lethal to fish.

The inhibition of brain ChE activity in fish may not be an important factor in the acute lethal effect produced by acephate. The important target organ(s) of acephate in lethal does may be peripheral rather than central in location. Fenitrothion, on the contrary, may produce the acute lethal effect through the inhibition of ChE in the central nervous system as a predominant factor and/or with the combination of peripheral tissue ChE inhibition.

Fenitrothion, at sublethal concentration, was found to produce greater ChE inhibition in the rat kidney, liver, erythrocytes and plasma than within the brain (Misu et al. 1966). Miyamoto (1977) who studied the absorption of radioisotope m-methyl-^{14}C fenitrothion at concentration of 0.02 mg/L in rainbow trout reported that after 6 hours of exposure the concentration of radiocarbon is highest in gall bladder and intestine, and after 24 hours the radiocarbon is present in nearly every tissue except brain and heart. These investigators studied the toxic effect at sublethal or very low levels of fenitrothion, therefore the rate of absorption and distribution might be different from that at the acute lethal level which was used in this study.

The skeletal muscle enzyme was not greatly affected and did not show much inhibition by either acephate or fenitrothion. The skeletal muscle ChE exhibited recovery and approached the normal level after 3 hours of exposure but decreased slightly after and tended to recover again at the end of experiments.

Schneider and Weber (1975) studied neuromuscular function and the ChE enzyme in the pectoral fin abdoctor muscle of largemouth bass (Micropterus salmoides) exposed to DFP (diisopropylfluorophosphate), an OP insecticide, and found that ChE in skeletal muscle of fish was not as important for neuromuscular transmission as has been established for the muscle of other vertebrates. They also suggested that the acute toxic effects of DFP to largemouth bass were not mediated by a collapse of neuroskeletal muscle function. According to this information and the result observed from this experiment, therefore, it can be concluded that ChE inhibition in skeletal muscle by acephate and fenitrothion is not the primary site or mechanism of action producing death in rainbow trout.

Enzyme inhibition in red blood cells and serum or plasma in fish exhibited a similar pattern with a fairly rapid decrease in enzyme activity during the first few hours after exposure, followed by a slower decrease or recovery of the enzyme activity. The recovery process may be caused by the regeneration of red blood cells in the bone marrow and the synthesis of new plasma enzyme in the liver as suggested by Gage (1967). The time required for recovery or regeneration of the enzyme will depend upon the degree of inhibition, the duration of exposure, the chemical structure of the inhibitors and the nature of enzyme involved (Gage, 1967).

The activity of blood ChE enzyme is generally regarded to be an index of exposure to OP insecticides and a separate determination on red blood cell and plasma has been suggested as having a greater diagnostic value than a determination on whole blood (Witter, 1963; Gage, 1967; Wills, 1972). Gage (1967) reviewed the literature on enzyme inhibition in the blood of mammals by several anti-ChE agents and suggested that the toxic effects are not likely to be encountered if the red blood cell and plasma enzyme activities remained above 50 and 25 percent respectively. In this experiment, using fenitrothion the enzyme activity of red blood cell and plasma was decreased to 28 and 12 percent of control respectively. However, the enzyme activity of red blood cell and plasma in acephate experiment remained high at 90 and 41 percent of control respectively. The higher enzyme activity in plasma may be due to the recovery of enzyme as described previously but the high enzyme activity in red blood cell may be due to the regeneration of red blood cell and/or the inability of acephate to penetrate into the red blood cell.

In general, ChE activity in serum or plasma of fish in this experiment exhibited more inhibition by both acephate and fenitrothion than red blood cell enzyme. Since plasma enzyme is more sensitive to both insecticides than red blood cell enzyme, it may be used to detect and indicate the exposure to OP insecticides in fish.

The inhibition of ChE in heart tissue by acephate and fenitrothion followed the same pattern as in serum enzyme with a rapid decrease in enzyme activity during the first few hours followed by a slower decrease or recovery of the enzyme activity. However, the heart ChE inhibition

did not correlate well with the decrease in heart rate. The heart tissue used in this study was the ventricle muscle which is not the primary control of the heart beat. Therefore, ChE inhibition in the heart ventricle muscle may only indicate the condition of the ventricle muscle activities such as the strength of contraction and electrical activity.

Inhibition of ChE in the gill tissue exhibited a pattern similar to serum enzyme (Figures 13 and 14). Gill enzyme activity in this experiment may be considered as the combination of enzyme from skeletal muscle and blood since the main structure of gills consists of both tissues and no attempt was made in this experiment to get rid of the blood that may be left in the gill filament when analysing the enzyme activity.

The result observed in this experiment showed that gill ChE is as sensitive as serum and heart to indicate exposure to OP insecticides. It is interesting to note that the gill is the major site of toxicant uptake since it has a relatively large surface area and is very permeable to most compounds. Therefore, acephate and fenitrothion should inhibit ChE enzyme that is present in the gill tissue before passing into the blood system and distributing to other tissues and organs of the fish body. The degree of change in the branchial vascular resistance and the pattern of blood flow through the gill filaments that take place during exposure to anti-ChE agents as suggested earlier is probably related to the inhibition of ChE in gill tissue, especially at the gill filaments. Specific experiments relating ChE inhibition and the increase in vascular resistance were not conducted in this study. Bergman et al.

(1974) reported that the decrease in level of C^{14}-urea influx and an increase in the perfusion pressure were correlated with concentration of ACh when applied from 10^{-8} to 10^{-6} moles/liter to isolated perfused trout gills.

It can be concluded, from these observations, that the determination of ChE activity in gill or a fraction of the gill, ie. gill filaments, may also provide a useful information to indicate the exposure to OP insecticides in fish.

2.3 Determination of serum electrolytes

The pattern of variations in serum electrolytes of rainbow trout associated with exposure to acephate and fenitrothion observed in this study indicates that both OP insecticides induced some degree of electrolyte imbalance in fish. The effects are characterized specifically by an increase in serum K^+ and a decrease in serum Cl^- concentrations. Several factors may contribute to these changes in serum electrolytes.

The gills of fish are not only the primary site of gas transfer between blood and water but also serve as an important pathway for extrarenal ion regulation (Conte, 1969). Changes that decrease or increase gas transfer rates may also affect ion and water exchange across the gill. There is a countercurrent arrangement of the flows of blood and water at the gill and ion and water diffusion across the gills can only be lowered by reducing the permeability and/or area of the respiratory epithelium (Randall et al. 1972). Recent histological examination of the trout gills revealed that most of the "mitochondria-rich cells" or "chloride cells" are located in the secondary lamellae, especially on

the afferent side (Morgan and Towell, 1973) and these cells are almost certainly the site of ionic transport (Conte, 1969; Maetz 1971). Therefore, the decrease in functional surface area and changes in the pattern of blood flow in the gill by acephate and fenitrothion as suggested previously may lead to a decrease in the rate of ion and water diffusion across the gill.

In freshwater teleosts, water tends to enter the body as the result of osmotic gradient across the gill and the intestine (Hickman and Trump, 1969), the skin of fish being impermeable (Bentley, 1962; Fromm, 1968), and water is eliminated through a single pathway, the kidney, in the form of urine. Discharge of copious urine necessarily involves a loss of salt dissolved in it, but this is compensated for by an active uptake of Na^+ and Cl^- through the gills (Richards and Fromm, 1970; Kerstetter and Kirschner, 1972) and by ionic exchange mechanisms (Evans, 1975). Fleming et al. (1962) who studied the effects of external salt concentrations and ChE inhibitors, eg. B.W. 284-C51 and Mipafox, on the gill ChE activity and sodium fluxes in several cyprinodontid fishes found an effect on sodium outflux only but not on sodium uptake. Koch (1954) however, has presented data which show that ChE is involved in the active uptake of sodium by the isolated gill of the crab (Eriocheir sinensis). It was suggested also by Silver (1974) that ChE may have some involvement in the process of permeability control and of transport in particular, the transport of sodium - across membranes eg. in erythrocyte and the blood brain barrier. Membrane permeability is influenced by pH and it is also possible that, in some instance, the pH of the system involved is altered when anti-ChE agents are administered (Silver, 1974). From these observations, it seems that role of ChE in the osmoregulatory

mechanisms in fish is still unclear and therefore it is not considered as a major factor in this study.

The mechanism that most likely caused the serum electrolyte changes in fish in this study is probably a shift in electrolyte concentrations among fluid compartments to maintain the electroneutrality due to the accumulation of certain metabolites. The results observed in cardiovascular and respiratory study as described previously suggested that both acephate and fenitrothion produced hypoxic symptoms in fish and some metabolites, ie. lactate, may increase due to an increase in anaerobic metabolism. Kirk (1974) who studied the effect of hypoxia on certain blood and tissue electrolytes of channel catfish (Ictalurus punctatus) reported that hypoxia produces an acidosis characterized by an increase in blood lactate and a decline in blood pH. He suggested that the changes in electrolyte concentrations which occurred were not due solely to osmoregulatory failure but were in part a response directed toward buffering acidic products generated by anaerobic metabolism.

Evidence from other studies in mammals indicated that as lactate ions accumulated during metabolic acidosis, levels of other anions (ie. Cl^- and HCO_3^-) declined to minimize any increase in total anion concentration (Tobin, 1958; Frisell, 1968). Several studies also indicated a decline in serum Cl^- and blood HCO_3^- in a variety of fishes following hypoxia (Black et al. 1962; 1966). The accumulation of lactate ions could also cause a concommitant increase in cations (ie. K^+ and Na^+) to maintain electroneutrality (Kirk, 1974). Glycogen mobilization and the exchange of glucose between the cellular and extracellular phases in fish is normally associated with potassium movement (Houston et al. 1971).

A linear relationship was observed between lactate production and K^+ loss in the heart of mammals due to ischemic conditions (Case et al. 1969; Gerlings et al. 1969). An increase in serum K^+ concentration observed in this study supported the condition of electrolyte disorder, hyperkalemia, in the heart tissues as demonstrated by the changes in ECG waveforms in the cardiovascular study.

SUMMARY AND CONCLUSION

This study can be summarized as follows;

1. Two organophosphorus (OP) insecticides; acephate (a phosphor--amidothioate with a basic structure of P=O), a relatively new OP insecticide with a very low toxicity to mammals; and fenitrothion (a phosphorothioate with a basic structure of P=S), a broad spectrum OP insecticide used extensively throughout the world for control of agricultural and forest pests, were tested on rainbow trout (Salmo gairdneri) fingerlings to study the effects of temperature stresses on acute lethality, and cholinesterase (ChE) inhibition in brain and skeletal muscle. Physiological responses of cardiovascular and respiratory systems, ChE inhibition in various tissues and changes in serum electrolytes in adult fish exposed to each insecticide were also observed to provide some more understanding on the sites of action producing death in fish.

2. Temperature stresses affected the acute lethality of each insecticide, as indicated by LC_{50} and MST values, but was more pronounced with fenitrothion than with acephate during the first 24 hour period. In general, fish died faster as temperature increased and slower as temperature decreased. The effects of temperature became less after 48 hours and no significant effects were observed after 96 hours of exposure. Temperature may influence the rate of uptake of each insecticide and the rate of biotransformation processes of fenitrothion by liver microsomal enzyme systems in fish. Fenitrothion, as an indirect ChE inhibitor probably requires biotransformation to fenitrooxon (having basic structure of P=O), which is a more potent ChE inhibitor. Acephate, however, probably does not require biotransformation since it has basic structure of P=O. In

the fenitrothion experiments, the average Q_{10} value of the rate of mortality of 3.3 and the changes in the slopes of mortality curves suggested that biotransformation processes occurred. In the acephate experiments, the observed Q_{10} values of the rate of mortality of 1.3 and the lack of differences in the slopes of mortality curves also suggested that temperature affected acephate toxicity by affecting the rate of absorption only.

3. Expressed as LC_{50} values (the concentration that produced 50 percent mortality), the toxicity of fenitrothion was about 600 to 1000 times greater than acephate, depending upon the test temperature. The difference in toxicity between acephate and fenitrothion may occur because of the difference in the ability of the chemicals to penetrate cell membranes and reach the biochemical target receptor (ChE) and in the capacity of the liver to activate fenitrothion to a more potent compound. Fenitrothion, as a lipid soluble compound, can penetrate cell membranes, be distributed within the fish body and be activated by microsomal liver enzyme system and react with target receptor more readily than acephate which is a lipid-insoluble compound.

4. There was no correlation between ChE inhibition levels in the brain and skeletal muscle and death of rainbow trout fingerlings exposed to acephate and fenitrothion at various concentrations. Dead fish, especially at higher insecticide concentrations, had a high ChE activity. Surviving fish, in some cases, had lower ChE activity than dead fish. Tissue homogenization used in the ChE analyses probably allows the intracellular ChE or nonfunctional enzyme pool, which may constitute about 80 percent of total enzyme, to become available to the substrate. Therefore there may be a high total ChE activity, but the extracellular

ChE or functional enzyme pool may be completely inhibited. Dead fish that stayed longer at lower insecticide concentrations exhibited low total ChE activity, since the insecticides had more time to penetrate cell membranes and to inhibit functional and nonfunctional enzyme pools in the tissues. Tolerance mechanisms may also develop and may allow surviving fish to adapt to low ChE levels and to the accumulation of ACh.

5. In adult rainbow trout, acephate and fenitrothion produced a decrease in heart rates, and increases in ventilation rate and amplitude which are similar to the characteristic of fish exposed to hypoxia. Hypoxic conditions are suggested to be caused by the anti-ChE action of these insecticides on the cardiovascular and respiratory systems in fish by increasing branchial vascular resistance and reducing the functional gill surface area. These conditions would interfere with the diffusion of gases across gill surfaces, decrease oxygen uptake and carbon dioxide excretion, resulting in the lack of oxygenated blood supply to tissues and organs in the body. Electrocardiogram (ECG) waveforms recorded during insecticide exposures were also compatible with myocardial ischemia (lack of oxygen supply to the heart tissue) and electrolyte disorders, ie. an increase in potassium, produced in fish heart. No other data were obtained in this experiment, however, to support this suggestion that OP insecticides induce internal hypoxia in fish.

6. Fenitrothion produced an increase in cough frequency, but acephate did not. Coughing is thought to be caused by gill irritation, but no evidence was obtained in this study to indicate that fenitrothion produced gill irritation. An alternative explanation has been provided by some investigators that coughing is controlled by neuronal activity

in the area of the hind brain. Fenitrothion, as a lipid soluble compound, may pass the blood-brain barrier and react with that part of the brain to affect coughing more readily than acephate which lacks the chemical properties for passing the blood-brain barrier.

7. Acephate and fenitrothion produced differential patterns of ChE inhibition in brain, gill, heart, red blood cell, serum and skeletal muscle in adult rainbow trout. The extent to which this enzyme was inhibited depends on the physicochemical properties as well as the disposition of both insecticides within the fish body. The validity of using brain ChE inhibition to indicate death of fish by OP insecticides, as suggested by several investigators, may be questioned, since the anti-ChE action of lipid-insoluble OP compounds, such as acephate, may be restricted to the periphery. Enzyme activities in the cardiovascular and respiratory systems, especially gills, heart and serum, were inhibited to a greater degree by each insecticide. It is suggested that these two systems are adversely affected by OP insecticides and therefore should be used to detect and indicate exposure to OP insecticides in fish.

8. Acephate and fenitrothion induced changes in serum electrolytes, characterized especially by an increase in serum K^+ and a decrease in serum Cl^- concentrations. These changes were considered to be caused by the movement of electrolytes among fluid compartments to maintain electro--neutrality. It is suggested that ChE may be involved in the maintenance of osmoregulatory process in fish but the evidence is not definitive and therefore cannot be considered as a major factor.

In order for a chemical (eg. an OP insecticide) to produce a

harmful effect on a biological system in fish, it must go to a specific receptor (ChE) of that system in proper form and dosage level. Thus the toxicological effects depend on the manner in which an agent is absorbed, its distribution within the system, whether it acts as such or requires some type of metabolic activation, the rate at which the agent is destroyed or inactivated, and the rate at which it is lost from the system. Since each of these processes (absorption, distribution, metabolism and excretion) is dependent on temperature, it is to be expected that toxicity would be influenced by temperature of the body as well as the environment. The effects of temperature on toxicity of indirect ChE inhibitors (ie. fenitrothion) is more complex than that of direct ChE inhibitors (ie. acephate) since at least two or more enzyme reactions are involved, ie. enzyme action in the activation and reaction with the target enzyme (ChE). The rate of toxicity of indirect ChE inhibitors is more dependent on temperature since the toxic action depends upon the activation product which results from biotransformation process.

The need for considering the interaction between insecticides and environmental factors, eg. temperature when determining the safe levels of such compounds to fish and other aquatic organisms is evident from this study. This study demonstrates that the application of acute lethality data of OP insecticides from cold or moderate temperatures to other environmental temperatures, eg. the tropical zone, requires consideration not only of the difference in fish species, water characteristics but also of the specific type of OP insecticides.

In order to protect fish from OP insecticide applications, field monitoring programs for assessing the effects of OP insecticides on fish

have to be developed. Inhibition of ChE in brain tissue has been suggested as an indicator in monitoring program by several investigators, however the results observed in this study indicated that it is not sensitve and is not correlated with the degree of poisoning. Toxic action of lipid insoluble compounds, eg. acephate, also indicated that its site of action is primarily on peripheral systems not central, therefore other tissues than brain may be better indicators of exposure. The cardiovascular and respiratory systems eg. gills, heart and serum were more sensitive to OP insecticides exposure.

From the results observed in this study, it is concluded that cardiovascular/respiratory systems of fish are very important sites of action of OP insecticides, especially acephate and fenitrothion, in rainbow trout. Further investigations, particularly the cardiovascular and respiratory changes in fish exposed to OP insecticides should be conducted to determine the possible effects of internal hypoxia induced by the ChE inhibition action on the vascular resistance in fish. Other investigations should be concentrated on the development of the ChE analysis methods to find the relationship between ChE inhibition levels in tissues of cardiovascular/respiratory systems and the OP insecticide exposure.

macromere surface and distinct regression of the LEMs and other micromeres towards the animal pole. This cap formation is not produced following colchicine treatment.

The polar lobe constriction mechanism, known to be dependent upon microfilament activity, was utilized as a model for testing the sensitivity of the filaments to certain tertiary amine local anesthetics, including tetracaine, procaine, and marcaine. All these compounds produced arrest and/or relaxation of the third polar lobe constriction when applied in high dosages. Both tetracaine and marcaine were demonstrated either to prevent the appearance of microfilament bundles during polar lobe formation or putatively to disrupt pre-existing bundles.

An experimental system was designed to test the hypothesis that observed LEM microfilaments may assist in intercellular adhesion, along with the septate desmosomes. The D macromere was removed mechanically from embryos pre-treated with cytochalasin B, colchicine, emetine, tetracaine, procaine, and marcaine. Failure of the LEMs to cover the wound cavity left by the detachment of D in the presence of certain drugs was taken as indirect evidence that the putative LEM filaments sensitive to these drugs were involved in the constrictive force directed towards the center of the embryo. Only cytochalasin B and marcaine induced this configuration. The microfilament-assisted adhesion hypothesis, therefore, was supported both by normal morphology and by inhibition experiments. The results supporting this hypothesis are significant in that they provide in vivo evidence concerning a functional role for microfilaments in numerous in vitro cell systems.

THE FUNCTIONAL SIGNIFICANCE OF VARIATION IN THE VOCAL COMMUNICATION SYSTEM OF JAPANESE QUAIL

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DAUGHERTY, Lynn Bayliss, Ph.D. University of Montana, 1977. 196pp. Director: Donald A. Jenni

This study attempted to elucidate a portion of the vocal communication system of Japanese quail by concentrating on the functional significance of variation within that system.

Ten note types were identified and described from sonagrams of recordings of vocalizations of sexually mature male and female Japanese quail made under a variety of laboratory conditions. Eight types of notes intermediate between pairs of note types and two types of notes intermediate between three note types were also identified and described.

Three types of variability were identified in the vocalizations of Japanese quail. "Normal variability" in which signal parameter values are distributed relatively normally around easily identifiable means fell near one end of a stereotypy-variability continuum and "continuous variability" in which values are distributed fairly evenly along a continuum fell near the opposite end. "Unit variability," in which highly stereotyped signal units are produced in varying combinations, represented a higher order of variability in the communication process.

Four vocalization types were identified and described on the basis of patterning of note types and types of variability present. Possible functions for these vocalization types were identified and related to the types of variability present.

Unit variability in the Japanese quail Male Whistle Vocalization was examined from two functional viewpoints, a psychophysiological hypothesis which was supported and a message-meaning hypothesis which was not supported by the findings. The distribution of Male Whistle Vocalization between series containing one note and those containing more than one note did not differ significantly for the variation types themselves but did differ significantly for prevalent versus non-prevalent variation types with non-prevalent variation types more common in the longer series.

The hypothesis that Crow Vocalizations from different unknown quail have differential meanings was tested experimentally. Under laboratory conditions male but not female Japanese quail responded differentially to different unknown male quail on the basis of their Crow Vocalizations alone. At least two signal characteristics, one of which was intensity, contributed to the differential meaning of the Crow Vocalization.

The results of this study suggest that variation as well as stereotypy can be an adaptive attribute of a communication system but that its functional significance is highly complex.

ORGANOPHOSPHATE INSECTICIDE TOXICITY IN RAINBOW TROUT (Salmo gairdneri). EFFECTS OF TEMPERATURE AND INVESTIGATIONS ON THE SITES OF ACTION

DUANGSAWASDI, Maitree, Ph.D. The University of Manitoba (Canada), 1977

In order to protect fish from organophosphorus (OP) insecticide applications, field monitoring programs for assessing the effects of OP insecticides on fish have to be developed. Detection of OP insecticide pollution in natural water requires knowledge of the sites of action and the effects of environmental factors on the toxicity of these chemical in fish. Two OP insecticides; acephate (a phosphoramidothioate and a direct inhibitor of cholinesterase [ChE]) and fenitrothion (a phosphorothioate and an indirect inhibitor of ChE), were tested on rainbow trout (Salmo gairdneri) fingerlings to study the effects of temperature stress on acute lethality and ChE inhibition in brain and skeletal muscle. Physiological responses of cardiovascular and respiratory systems, ChE inhibition in various tissues and changes in serum electrolytes in adult fish exposed to each insecticide were observed to provide some more understanding on the sites of action of OP insecticide producing death in fish.

Temperature stress affected the acute lethality of each insecticide but was more pronounced with fenitrothion than with acephate during the first 24 hour period. The effects of temperature stress became less after 48 hours and no significant effects were observed after 96 hours of exposure. Expressed as LC_{50} values (the concentration that produced 50 percent mortality), the toxicity of fenitrothion was about 600 to 1000 times greater than acephate depending upon test temperature. There was no correlation between ChE inhibition levels in the brain and skeletal muscle of rainbow trout fingerlings and the concentration of acephate and fenitrothion which produced mortality.

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ORGANOPHOSPHATE INSECTICIDE TOXICITY IN RAINBOW TROUT (SALMO GAIRDNERI). EFFECTS OF TEMPERATURE AND INVESTIGATIONS ON THE SITES OF ACTION.
DUANGSAWASDI, MAITREE (PH.D. 1977 THE UNIVERSITY OF MANITOBA (CANADA)
).
PAGE 5228 IN VOLUME 38/11-B OF DISSERTATION ABSTRACTS INTERNATIONAL.

macromere surface and distinct regression of the LEMs and other micromeres towards the animal pole. This cap formation is not prevented following colchicine treatment.

The polar lobe constriction mechanism, known to be dependent upon microfilament activity, was utilized as a model for testing the sensitivity of the filaments to certain tertiary amine local anesthetics, including tetracaine, procaine, and marcaine. All these compounds produced arrest and or relaxation of the third polar lobe constriction when applied in high dosages. Both tetracaine and marcaine were demonstrated either to prevent the appearance of microfilament bundles during polar lobe formation or putatively to disrupt pre-existing bundles.

An experimental system was designed to test the hypothesis that observed LEM microfilaments may assist in intercellular adhesion, along with the septate desmosomes. The D macromere was removed mechanically from embryos pre-treated with cytochalasin B, colchicine, emetine, tetracaine, procaine, and marcaine. Failure of the LEMs to cover the wound cavity left by the detachment of D in the presence of certain drugs was taken as indirect evidence that the putative LEM filaments sensitive to these drugs were involved in the constrictive force directed towards the center of the embryo. Only cytochalasin B and marcaine induced this configuration. The microfilament-assisted adhesion hypothesis, therefore, was supported both by normal morphology and by inhibition experiments. The results supporting this hypothesis are significant in that they provide in vivo evidence concerning a functional role for microfilaments in numerous in vitro cell systems.

THE FUNCTIONAL SIGNIFICANCE OF VARIATION IN THE VOCAL COMMUNICATION SYSTEM OF JAPANESE QUAIL
Order No. 7806227

DAUGHERTY, Lynn Bayliss, Ph.D. University of Montana. 1977. 196pp. Director: Donald A. Jenni

This study attempted to elucidate a portion of the vocal communication system of Japanese quail by concentrating on the functional significance of variation within that system.

Ten note types were identified and described from sonagrams of recordings of vocalizations of sexually mature male and female Japanese quail made under a variety of laboratory conditions. Eight types of notes intermediate between pairs of note types and two types of notes intermediate between three note types were also identified and described.

Three types of variability were identified in the vocalizations of Japanese quail. "Normal variability" in which signal parameter values are distributed relatively normally around easily identifiable means fell near one end of a stereotypy-variability continuum and "continuous variability" in which values are distributed fairly evenly along a continuum fell near the opposite end. "Unit variability," in which highly stereotyped signal units are produced in varying combinations, represented a higher order of variability in the communication process.

Four vocalization types were identified and described on the basis of patterning of note types and types of variability present. Possible functions for these vocalization types were identified and related to the types of variability present.

Unit variability in the Japanese quail Male Whistle Vocalization was examined from two functional viewpoints, a psychophysiological hypothesis which was supported and a message-meaning hypothesis which was not supported by the findings. The distribution of Male Whistle Vocalization between series containing one note and those containing more than one note did not differ significantly for the variation types themselves but did differ significantly for prevalent versus non-prevalent variation types with non-prevalent variation types more common in the longer series.

The hypothesis that Crow Vocalizations from different unknown quail have differential meanings was tested experimentally. Under laboratory conditions male but not female Japanese quail responded differentially to different unknown male quail on the basis of their Crow Vocalizations alone. Among two signal characteristics, one of which was intensity, only attributed to the differential meaning of the Crow Vocalizations.

The results of this study suggest that variation as well as stereotypy can be an adaptive attribute of a communication system but that its functional significance is highly complex.

ORGANOPHOSPHATE INSECTICIDE TOXICITY IN RAINBOW TROUT (Salmo gairdneri). EFFECTS OF TEMPERATURE AND INVESTIGATIONS ON THE SITES OF ACTION

DUANGSAWASDI, Maitree, Ph.D. The University of Manitoba (Canada). 1977

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THESIS Ph.D., 1978

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